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Complete List of Authors:	Li, Ping; Peking university, Department of Occupational and Environmental Health Science Gu, Yongen; Peking university, Department of Occupational and Environmental Health Science Yu, Shanfa; Institute of Occupational Medicine, Department of Occupational Health Science Li, Yang; Peking university, Department of Occupational and Environmental Health Science Yang, Jinglin; Peking university, Department of Occupational and Environmental Health Science Jia, Guang; Peking university, Department of Occupational and Environmental Health Science
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The suitable condition of 8-OHdG and micronucleus as genotoxic damage biomarkers in occupational chromate exposed workers

Ping Li[1], Yongen Gu*[1], Shanfa Yu [2], Yang Li[1], Jinglin Yang[1], Guang Jia[1]

* co-author: equally with the first author

1. Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

2. Department of Occupational Health Science, Institute of Occupational Medicine, Zhengzhou City, Henan Province 450052, P. R. China

Address correspondence to:

Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

Tel: +86-010-8280-2333, Fax: +86-010-8280-2333

E-mail: jiaguangjia@bjmu.edu.cn

Abstract

Objectives: We aimed to investigate the suitable condition of 8-hydroxy-2' -deoxyguanosine (8-OHdG) and micronucleus(MN) at different levels of occupational chromate exposure.

Design: A cross-sectional study was used.

Participants: 84 workers exposing chromate at least one year were chosen as exposed group, while 30 non-exposed individuals were used as controls.

Main outcome measures: Environmental and biological exposure of chromate was assessed respectively by measuring the concentration of chromate in the air (CrA) and blood (CrB) by ICP-MS in all subjects. CBMN was conducted, in which many indexes including MNCC, MNC, NPB and NBUD were calculated, while the urinary 8-OHdG was measured by ELISA method and normalized by the concentration of Cre.

Results: Compared with the control group, the levels of CrA, CrB, MNCC, MNC and 8-OHdG in chromate exposed group were all significantly higher (P<0.05). There was a positive correlation between log(8-OHdG) and LnMNCC or LnMNC (r=0.377 and r=0.362). The levels of LnMNCC , LnMNC and Log (8-OHdG) did not have the simple linear relationship with the concentration of CrB. There was a significantly positive correlation between Log (8-OHdG) and CrB when CrB level was below 10.50 ug/L (r=0.355). While a positive correlation was found between LnMNCC or LnMNC with CrB when CrB level was lower than 9.10 ug/L(r=0.365 and r=0.269 respectively).

Conclusions: The MN and 8-OHdG can be used as the biomarker for the genetic damage in chromate exposed group, but only when CrB levels were lower than 9.10ug/L and 10.50ug/L respectively, they can accurately reflect the degree of genetic damage.

Key terms: chromate; Genetic damage; 8-OHdG; Micronucleus, Concentration of chromate in the blood

Abbreviation:

Cytokinesis-block micronucleus test-----CBMN

Micronuclei cell count ------MNCC

Micronucleus count -------MNC

Nuclear bridge------NPB

Nuclear bud --------NBUD

Creatinine------Cre

Strengths and limitations of this study(Article summary)

Strengths: All our results had provided new insight that only when the concentration of CrB was lower than 9.10ug/L and 10.45ug/L respectively, the MN and 8-OHdG can be used as the effective biomarkers to show the degree of genetic damage for the chromate occupational exposure, otherwise, above these levels, the cytotoxic effects might play an important role and the fate of cells with serious genetic damages may turn into apoptosis or necrosis, consequently which could lead to the false appearance of lower degree of MN and 8-OHdG at higher chromate exposed level.

Limitations: the size of this study population was not very large, especially the group of control individuals, so we chose some references to give the normal value of MN and 8-OHdG in other controls to reduce the mitigation of bias. In this research, we got the recommended condition of 8-OHdG and MN as genetic damage biomarker in chromate exposed group. It is necessary that more occupational epidemiological survey in different chromate producing factories should be chosen to verify this conclusion.

Introduction

Chromate is a widely used chemical in industrial and agricultural production in china, which could generate many pollutants including the waste water, waste gas and waste residue in chromate production, usage, transportation and storage. Long-term chromate exposure in occupational workplace can affect the health status of workers even cancer, so it has been declared as a well-known environmental and occupational hazards.

There are many different valences of chromate, in which the hexavalent chromate (Cr-VI) is the most harmful one. Cr-VI can enter human body mainly by inhalation during occupational activities. When entering into respiratory system (nose, bronchial and lung), some Cr-VI could be converted to Cr-V, Cr-IV and Cr-III, and accumulated in bronco-alveolar lining fluid, mucosa and pulmonary tissues[1,2]. This transformation also can consequently form many reactive intermediates with oxidative stress[3,4]. Both Cr-III and ROS could contribute to interact with various proteins, DNA and other biological macromolecule to cause damage on DNA and chromosome, which can initiate carcinogenesis if accumulated to some degree[5, 6, 7].

Based on the evidence above, 8-OHdG as a product of oxidative DNA damage, can be used to assess the oxidative damage and DNA mutations that are induced by the ROS in clinical[8], environmental[9,10] and occupational setting in vitro[11,12]or in cell cultures [13]. MN in peripheral blood originates from chromosome fragments that are attacked by certain physical and chemical factors such as chromium or whole chromosomes that lag behind at anaphase during nuclear division. When the excision-repairable DNA lesions induced in G0/G1 phase, they can be converted to MN by using inhibitors of the gap filling step of excision repairmen, so that unfilled gaps are converted to double strand breaks after S phase[14], so the frequency of MN can be used to reflect the genetic damage[15]. The CBMN test is a common method to detect the MN frequencies including many indexes such as MNCC, MNC, NPB and NBUD. In these indexes, MNCC and MNC were commonly used to detect the DNA damage[14] .

Previous studies have chose the relationship between CrB and urinary 8-OHdG or MN to discuss the feasibility of 8-OHdG and MN as the biomarker for chromium exposure, however, the conclusion about this connection have yielded conflicting results: Kuo found the linear correlation between urinary 8-OHdG and CrB[16], but Gao, Kim and Zhang didn't confirm it subsequently[17,18,19], many studies had been identified there was some association between CrB and MN frequencies[20,21,22], but the suitable conditions and limitations for 8-OHdG and MN as biomarkers for occupational chromate exposure have still been unclear.

So our researches aimed to observe the effect of chromate exposure on genetic damage in occupational workers, especially urinary 8-OHdG for the oxidative DNA damage and MN for chromosome damage. Then it was discussed whether MN frequency and 8-OHdG can be used as the effective genotoxic biomarkers at different levels of chromate exposure.

Materials and methods

Study design and population

A cross-sectional survey was designed for this research. The factory was chosen as the work place in Henan province in china because (1) the product--potassium dichromate was relatively simple, most of which was water-soluble hexavalent chromate. (2) Annual health check-ups were offered in this factory for workers, which allowed us to easily collect specimens from workers to minimize the interference with normal work schedules.

In this research, 84 workers exposed to chromate in the factory were chosen as exposed group, while 30 non-exposed individuals working in the administration office were as control group. The criteria of subjects included: (1) workers in exposed group were at least one year employment and 3 months working in the same work position. (2) aged between 25-50. (3) no medical history of allergy, asthma or allergic rhinitis, (4) all subjects with skin infections, fever or other clinical diseases should be excluded during the sampling period. (5) Pregnant and nursing women were not

enrolled. .

All subjects were in the same factory with similar education and social backgrounds. They all were requested to complete a questionnaire and had a clinical examination. The questionnaire included a lot of information including occupational history, personal medical history, medication used in 4 weeks before the study, body weight and height, hair dye, house decoration, radiation exposure, individual protection, smoking status and alcohol intake.

Ethical consideration

This study was approved by Medical Ethics Committee of Peking University, Health Science Center (HSC), Beijing, China. Written informed consent from each study subject was also obtained.

Air and Biological exposure assessment

Six air sampling sections were respectively chosen in the workplaces of two groups, 10 sampling points were chosen in each section. The sampling process was used pumping at 1L/min for 8 hours (Sp730, TSI Corporation, USA), the membranes used in this study were MCE mixed cellulose ester filters (Φ37mm, pall, America).The average concentration of all sampling points on the membranes in the same group was measured by atomic absorption spectrometry and then calculated to evaluate the CrA during the whole production process, the detection limit for the CrA was 0.001 μg/L.

2ml anticoagulant (EDTA and heparin) blood in peripheral was drew from each subject after finishing the questionnaire and then assigned to two tubes on average. They were respectively used to measure the CrB and CBMN. The concentration of CrB was measured by inductively coupled plasma mass spectrometer (ICP-MS)[26]. the detection limits was 0.0012 μg/L. The tubes used above had been detected their background value before research to ensure less elements and heavy metals contamination. .

At the end of the work-shift, 30mL urine sample of each subject was collected into a 50 mL metal-free polypropylene centrifuge tube (Falcon, BD Biosciences) and

stored at -80℃ until used to measure the contents of 8-OHdG and Cre.

Determination of urinary 8-OHdG

According to the manufacturer's instructions, urine samples were firstly centrifuged at 1500 rpm for 7 min, secondly the supernatant was chosen to determine the content of urinary 8-OHdG using ELISA kit (USA Cayman chem, USA Cayman chem, 8-OHdG EIA kit) by Multiskan MK3 (Thermo, USA) and Cre by alkaline picric acid assay with a commercial kit (Ausbio Laboratories Co., Ltd. China) using a Hitachi 7170A automatic analyzer (Hitachi Corp, Japan). The results of 8-OHdG were regulated by Cre to avoid the potential interference of different urine density among the subjects.

CBMN test

Peripheral venous blood which was designed in heparinized tubes with individual numbers was taken to measure the MN frequency. The indexes (MNCC, MNC, NPB and NBUD) were counted in 1000 binuclear lymphocytes of each individual according to Fenech's protocol[23]. All scoring was carried out by two independent researchers through double - blind method. If the result from these two researchers was different, another researcher should be asked to verify the scores.

Statistical analysis

Epidata 3.0 software was used to entry the questionnaire and experimental data into computer. The whole process was utilized double-entry and logistical error check to ensure the accuracy.

All analysis was performed with SPSS16.0. Normality was assessed by Kolmogorov-Smirnov (*K-S*) test, the variables including 8-OHdG, MNCC and MNC did not meet the normality, so Log or Ln transformation was made for normality approximation. Continuous and categorical parameters between chromate exposed group and control group were tested using the two sample *t* test (or Mann-Whitney U nonparametric test) and χ^2 test. Curving correlation and linear regression were performed. Statistical significance was for two-sided. The *P* values were defined as α<0.05.

Results

General information analysis

A total of 114 subjects including 84 chromate exposed workers (mainly in form of $K_2Cr_2O_7$) and30 controls were recruited in this study. The working age of chromate exposed group was (7.82 ± 5.51) years. Furthermore, the personal protection (gloves and masks) of workers was above 90%. The demographic characteristics of all subjects in this research were presented in Table 1, which showed that there were no significant differences in the distribution of gender, age, smoking and alcohol consumption between the two groups.

Table 1 General information of chromate exposed group and control group

	Group	Exposed group (n=84)	Control group (n=30)	*T*	χ^2	*P*
Age	$\overline{X}$±S	35.73 ±7.85	34.83 ± 8.83	0.432		0.666
≤35	n (%)	40 (47.62)	17 (56.67)		0.187	0.493
>35		44 (52.38)	13 (43.33)			
Gender	n (%)					
Male		62 (73.81)	19 (63.33)		0.869	0.351
Female		22 (26.19)	11 (36.67)			
Smoke	n (%)					
Yes		30 (35.71)	8 (26.67)		0.610	0.435
No		54 (64.29)	22 (73.33)			
Alcohol	n (%)					
Yes		29 (34.52)	16 (53.33)		2.530	0.092
No		55 (65.48)	9 (46.67)			
8-OHdG (ug/g Cre)	$\overline{X}$±S	43.76±34.89	27.21±13.76	3.354		<0.001
MNCC (‰)	M(Q)	6.00 (4.00)	3.20 (2.10)	2.420		0.004
MNC (‰)	M(Q)	7.40 (4.47)	3.74 (2.94)	3.401		0.001
NBUD (‰)	M(Q)	1.11 (1.20)	1.16 (1.17)	0.163		0.871
NPB (‰)	M(Q)	1.28 (1.15)	1.42 (1.34)	0.476		0.635

Note: Smoking referred to suck at least one cigar per day and last one year or more, smoking quit but ess than one year was also included. Alcohol was definitive by weekly drinking no less than three times

Concentration of CrA and CrB

As was shown in Figure 1, the concentration of CrA in chromate exposed group

[(15.45±19.00) μg/m^3] was much higher than that in control group [(0.23±0.38) μg/m^3](P<0.001), but still under the exposure limitation of chromate [(50μg/m^3)(2012, ACGIH)]. The levels of CrB in chromate exposed group [(9.45± 9.47) μg/L] were also significantly higher than that in control group [(4.05±1.87) μg/L] (P<0.001).

Levels of urinary 8-OHdG, and serum CBMN indexes

As for the data distribution of indexes such as 8-OHdG, MNCC, MNC, NBUD and NPB (Table 1), there were three kinds of indexes (8-OHdG, MNCC and MNC) . They were higher in chromate exposed group than that in control group (P<0.05), which showed that 8-OHdG, MNCC and MNC could be used as the genetic damage biomarkers feasibly caused by chromate exposure.

Correlation

As two biomarkers for genetic damage, there was a positive correlation between log (urinary 8-OHdG) and LnMNCC and LnMNC (r=0.377 and r=0.362 respectively) P<0.05)(Figure 2).

Correlation was analyzed between the concentration of urinary 8-OHdG and CrB. As was recorded in Figure 3, there was no linear correlation but a curve fitting between CrB and Log (8-OHdG), we found the value of 8-OHdG was decreased when the concentration of CrB was more than 10.50μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high exposed group (CrB ≥10.50μg/L) and the low exposed group (CrB <10.50μg/L). A positive correlation was shown between CrB and log (8-OHdG) when the CrB Level was lower than10.50μg/L (r=0.355, P<0.05), while there was a negative correlation between CrB and log (8-OHdG) when the CrB Level was higher than10.50μg/L.

The relationship between the concentration of MNCC or MNC and CrB was also analyzed in this research (Figure 4). there were no linear correlation but a curve fitting between CrB and LnMNCC or LnMNC. We found the value of MNCC or MNC was decreased when the concentration of CrB was more than 9.10μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high

exposed group (CrB ≥9.10μg/L) and the low exposed group (CrB <9.10μg/L). A positive correlation was shown betweenCrB and LnMNCC or LnMNC in higher chromate exposed group (r=0.365 and r=0.269 respectively, P<0.05), while a significantly negative relationship was found between CrB and LnMNCC or LnMNC in the lower chromate exposed group.(r=-0.279 and r=-0.261 respectively, P<0.05).

Discussion

Long-term and low level chromate exposure can not only increase the body's internal load but also cause a variety of harmful effects on workers' health even increase the incidence of human cancer [24, 25]. In occupational activity, chromate could enter into workers' body mainly by respiratory system, then be metabolism and excreted by urine, so our previous researches have proved the concentration of chromium in whole blood and urine can be used as the indicators to assess chromate biological exposure[6, 26]. In this study, it was found that the concentrations of CrA and CrB in exposed group were all significantly higher than that in control group (P<0.05) ,while the CrB level in chromate exposed group was nine times more than that in the general population of our country (1.19 μg/L)[27], which showed that the conclusion was credible by contrasting the genetic damage indexes between chromate exposed group and control group.

As we all known when Cr-VI enters human blood, it will be converted into other valence chromate compounds such as Cr-III, which could not only produce a large amount of ROS that can cause the redox system imbalance, but also directly or indirectly coordinate with DNA or protein. The transformation above can affect genetic stability including oxidative DNA lesion, DNA cross-links, single and double strand breaks and so on[28,29,30]. Besides, long-term chromate exposure can also cause cyto-toxicity to lead cells apoptosis[31].

Urinary 8-OHdG has been demonstrated as a biomarker for oxidative DNA damage in chromate exposure not only by animal experiment but also by many

epidemiological researches[32,33], because it is the site that ROS often attacks, but the dose-response relationship with occupational exposure indicators was not depicted clearly. Overall, in this research, the relationship between urinary 8-OHdG and CrB was two-way changes. The concentration of urinary 8-OHdG was not significantly increasing when the level of CrB is more than 10.50μg/L, which showed that when the CrB was higher than some degree, the concentration of urinary 8-OHdG would fail to predict the degree of DNA damage. There are some reasons for this result, firstly Sumner E.R has proved that oxidative DNA damage by chromate exposure mainly target on specifically certain glycol tic enzymes on 8-OHdG[34], which means once higher burden of CrB was produced, it could oxidize and change the structure of glycolytic enzymes to reduce the production of free 8-OHdG. secondly, some researchers have proved that 8-OHdG was not the final product of redox reaction, so when the level of CrB is higher, 8-OHdG could be converted to these further oxidation products such as Spiroiminodihydantoin (Sp)[35], so the concentration of free 8-OHdG was reduced. Thirdly, as we all know, the kidney can be damaged by chronic chromate exposure. As the level of CrB increased, the kidney was at greater health risk, which ultimately affect the exertion of 8-OHdG in urine[36].

Micronuclei is another biomarker which commonly used as the genetic damage of chromate exposure[37,38]. Many researches showed that chromate exposure could cause the increasing of MN frequency in cell research, animals experiment, and human being[39,40]. In this research, the similar conclusion was proved that the MN frequency was significantly higher in chromate exposed group than that in control group and the general population[41](P<0.05). The frequency of MNCC and MNC (as the sensitive indexes of MN) has statistical differences between these two groups. However, the dose-response relationship with CrB still needs many further investigations, so this research was meaningful to discuss the suitable condition of MN as genetic damage. In this study, no linear correlation was shown between CrB and LnMNCC or LnMNC, the MN frequency did not increase as the CrB elevated. When all subjects were divided into two subgroups by the concentration of CrB, a positive correlation was shown between CrB and LnMNCC and LnMNC in high CrB

group(CrB≥9.10μg/L) (r=0.365 and r=0.269 respectively), while a significantly inverse relationship was found between CrB with LnMNCC and LnMNC in low CrB group(CrB<9.10μg/L) (r=-0.279 and r=-0.261 respectively). The reason for these results may be that the occupational chromate exposure can increase the frequency of the MN in some range. As the increasing of CrB, more serious genotoxic damage should be caused such as the apoptosis or necrosis of lymphocyte, which contrarily decrease the frequency of MN in this situation[42,43] .

Both MN and urinary 8-OHdG can be used to predict different types of DNA damage. A positive correlation between urinary 8-OHdG and MN was found, which suggested that these two indicators as genetic damage biomarker can be verified each other.

Conclusions

All these above had provided new insight that both MN and urinary 8-OHdG can be used as the genetic damage biomarkers caused by occupational chromate exposure at some leve, the combination of these indicators can improve the credibility of the results. It is not the simple linear relationship between their concentration and the level of chromate exposure. Only when the level of CrB is below 9.10μg/L and 10.50μg/L, the MN frequency and urinary 8-OHdG can show the degree of genetic damage respectively produced by chromate exposure quantitatively.

Contributor ship statement

All the authors included in the paper grant the criteria of the authorship. PL together with YG conceived and executed this investigation, analyzed of the data and described this manuscript. YL and JY supported this investigation from the epidemiological aspect. GJ is responsible for the whole conduct of this study and all

content of this. SFY contribute to the organization and arrangement for the scene investigation. All authors commented critically on the manuscript and agreed with this submitting.

Competing interests

There are not competing interests.

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Data sharing

There is not data sharing.

Reference

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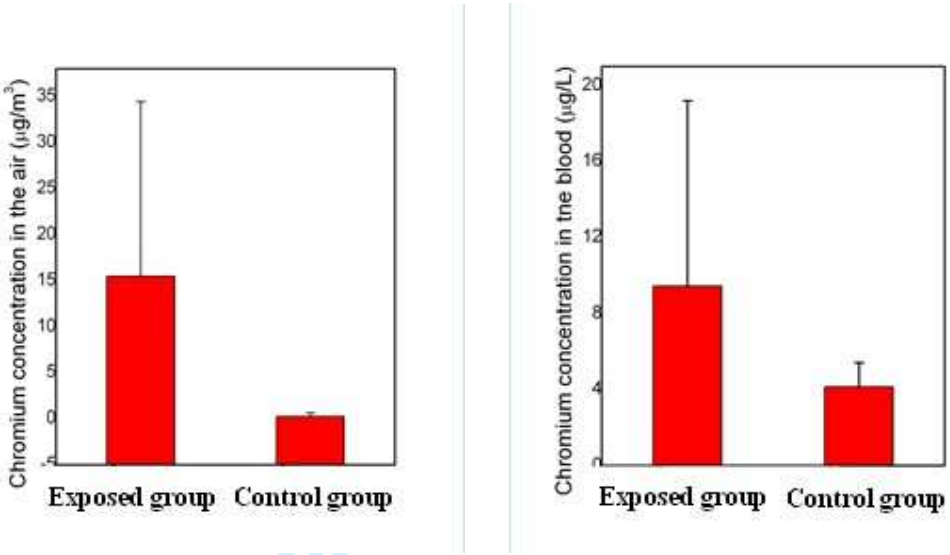


Figure1 The concentration of CrA and CrB in chromate exposed group and control group

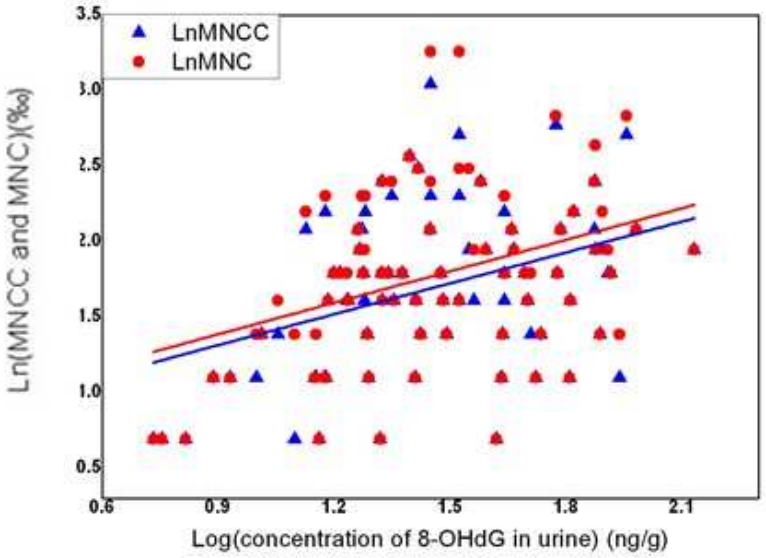
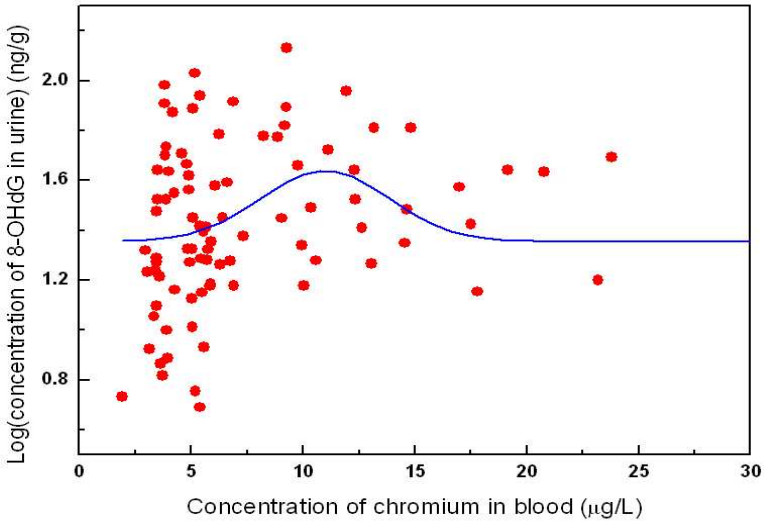
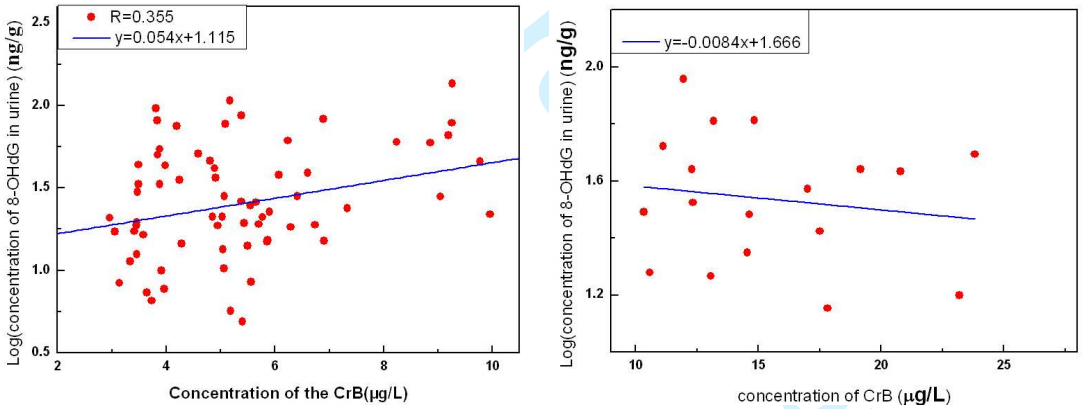


Figure 2 Correlation between MNCC and MNC with the concentration of 8-OHdG in urine in exposed group



a



b c

Figure3 The concentration between urinary 8-OHdG and CrB, a means the Curve fitting between urinary 8-OHdG and CrB in chromate exposed group, b means the concentration between urinary 8-OHdG and CrB in lower chromate exposed group(CrB<10.50), c means the concentration between urinary 8-OHdG and CrB in higher chromate exposed group(CrB≥10.50).

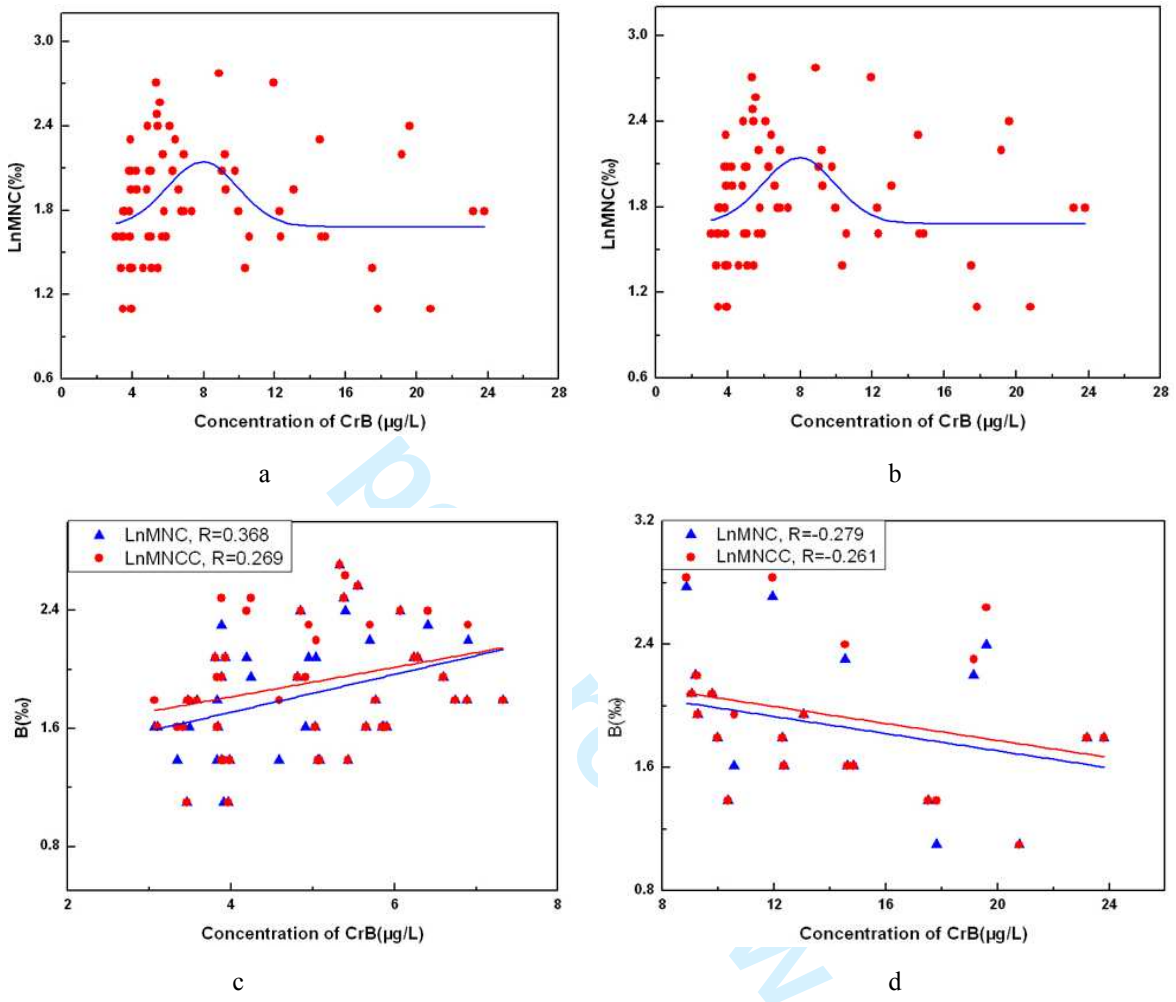


Figure4 The concentration between LnMNC or LnMNCC and CrB, a means the Curve fitting between LnMNC and CrB in chromate exposed group, b means the Curve fitting between LnMNCC and CrB in chromate exposed group, c means the concentration between LnMNC or LnMNCC and CrB in lower chromate exposed group(CrB<9.10), d means the concentration between LnMNC or LnMNCC and CrB in higher chromate exposed group(CrB≥9.10).

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A cross-sectional study: the suitable condition of 8-OHdG and micronucleus as genotoxic biomarkers in chromate exposed workers

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A cross-sectional study: the suitable condition of 8-OHdG and micronucleus as genotoxic biomarkers in chromate exposed workers

Ping Li[1], Yongen Gu*[1], Shanfa Yu [2], Yang Li[1], Jinglin Yang[1], Guang Jia[1]

* co-author: equally with the first author

1. Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

2. Department of Occupational Health Science, Institute of Occupational Medicine, Zhengzhou City, Henan Province 450052, P. R. China

Address correspondence to:

Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

Tel: +86-010-8280-2333, Fax: +86-010-8280-2333

E-mail: jiaguangjia@bjmu.edu.cn

Key terms: Chromate; Genotoxic; 8-hydroxy-2'-deoxyguanosine; Micronucleus; Concentration of chromate in the blood.

Abstract

Objectives: We aimed to investigate the suitable condition of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and micronucleus (MN) as genetoxic biomarkers at different levels of occupational chromate exposure.

Design: A cross-sectional study was used.

Participants: 84 workers who exposed chromate at least one year were chosen as exposed group, while 30 non-exposed individuals were used as controls.

Main outcome measures: Environmental and biological exposure of chromate was assessed respectively by measuring the concentration of chromate in the air (CrA) and blood (CrB) by ICP-MS in all participants. CBMN including MNCC, MNC, NPB and NBUD were calculated, while the urinary 8-OHdG was measured by ELISA method and normalized by the concentration of Cre.

Results: Compared with the control group, the levels of CrA, CrB, MNCC, MNC and 8-OHdG in chromate exposed group were all significantly higher (P<0.05). There was a positive correlation between log(8-OHdG) and LnMNCC or LnMNC (r=0.377 and r=0.362). The levels of LnMNCC , LnMNC and Log (8-OHdG) all have parabola correlation with the concentration of CrB. But there was a significantly positive correlation between Log (8-OHdG) and CrB when CrB level was below 10.50 ug/L (r=0.355), while a positive correlation was also found between LnMNCC or LnMNC with CrB when CrB level was lower than 9.10ug/L (r=0.365 and r=0.269 respectively).

Conclusions: The MN and 8-OHdG can be used as the genotoxic biomarkers in chromate exposed group, but only when CrB levels were lower than 9.10ug/L and 10.50ug/L respectively, they can accurately reflect the degree of genetic damage.

Strengths and limitations of this study (Article summary)

Strengths: All our results had provided new insight that only when the concentration of CrB was lower than 9.10ug/L and 10.45ug/L respectively, the MN and 8-OHdG can be used as the effective biomarkers to show the degree of genetic damage in chromate exposed group. Otherwise, when the concentration of CrB was above these levels, the cytotoxic effect might play an important role and the cells with serious genetic damage may turn into apoptosis or necrosis, consequently which could lead to the false appearance of lower degree of MN and 8-OHdG.

Limitations: the sample size of this study was not very large, especially the control group, so we chose some references to give the value of MN and 8-OHdG in normal population to reduce the generation of bias. And it is necessary that more sample size epidemiological surveys in different chromate producing factories should be chosen to verify our conclusion.

Abbreviation:

Cytokinesis-block micronucleus test-----CBMN

Micronuclei cell count ------MNCC

Micronucleus count -------MNC

Nuclear bridge------NPB

Nuclear bud --------NBUD

Creatinine------Cre

Introduction

Chromate is a widely used chemical in industrial and agricultural production in china, which could generate many pollutants including the waste water, gas and residue in its production, usage, transportation and storage. Previous studies had proved that long-term chromate exposure in occupational workplace could affect the health status of workers even cancer, so it has been declared as a well-known environmental and occupational hazards[1].

There are many different valences of chromate, in which the hexavalent chromate (Cr-VI) is the most harmful one. Cr-VI can enter human body mainly by inhalation during occupational activities. When entering into respiratory system (nose, bronchial and lung), some Cr-VI could accumulate in bronco-alveolar lining fluid, mucosa and pulmonary tissues[1,2] and then cross the cell membrane through non-specific phosphate/ sulfate anionic transporters to the blood. This transformation also can consequently form many reactive intermediates (Cr-V, Cr-IV and Cr-III) and reactive oxygen species (ROS) with oxidative stress[3,4]. Both Cr-III and ROS could contribute to interact with various biological macromolecule such as Cr-DNA adducts, Cr-protein adducts, and protein-Cr-DNA adducts. This can cause damage on DNA and chromosome including base modification, single-strand breaks and double-strand breaks. These changes can result in genetic damage and ultimate carcinogenesis if accumulated to some degree[5, 6, 7].

Based on the evidence above, many studies have proved that 8-OHdG can be used as a biomarker to assess the oxidative damage and DNA mutations that are induced by the ROS in clinical[8], environmental[9,10] and occupational setting in vitro[11,12]or in cell cultures [13]. MN in peripheral blood was another biomarker to show the genetic damage. It originates from chromosome fragments that are attacked by certain physical and chemical factors such as chromate or whole chromosomes that lag behind at anaphase during nuclear division. When the excision repairable DNA lesions induced in G0/G1 phase, they can be converted to MN by using inhibitors of

the gap filling step of excision repairmen, so that unfilled gaps are converted to double strand breaks after S phase[14], so the frequency of MN can be used to reflect the genetic damage[15]. The CBMN test is a common method to detect the MN frequencies including many indexes such as MNCC, MNC, NPB and NBUD. In these indexes, MNCC and MNC were commonly used to detect the DNA damage[14] .

Previous studies have discussed the relationship between CrB and urinary 8-OHdG or MN in chromate exposed group to investigate the feasibility of 8-OHdG and MN as genetic damage biomarkers. However, the conclusion about this connection have yielded conflicting results: Kuo found the linear correlation between urinary 8-OHdG and CrB[16], but Gao, Kim and Zhang all didn't confirm it subsequently[17,18,19], some research had been identified there was some association between CrB and MN frequencies[20,21,22], but the suitable conditions and limitations of 8-OHdG and MN as genotoxic biomarkers for occupational chromate exposure have still been unclear So our researches aimed to observe the effect of chromate exposure on genetic damage in occupational workers, especially urinary 8-OHdG for the oxidative DNA damage and MN for chromosome damage. Then we discussed whether MN frequency and 8-OHdG can be used as the effective genotoxic biomarkers at different levels of chromate exposure.

Materials and methods

Study design and population

A cross-sectional survey was designed for this research. The factory was chosen as the work place in Henan province in china because (1) the product--potassium dichromate was relatively simple, most of which was water-soluble hexavalent chromate. (2) Annual health check-ups were offered in this factory for workers, which allowed us to easily collect specimens to minimize the interference with normal work schedules.

In this research, 84 workers exposed to chromate in the factory were chosen as exposed group, while 30 non-exposed individuals working in the administration office were as control group. The criteria of subjects included: (1) workers in exposed group were at least one year employment and 3 months working in the same work position. (2) aged between 25-50. (3) no medical history of allergy, asthma or allergic rhinitis, (4) all subjects with skin infections, fever or other clinical diseases should be excluded during the sampling period. (5) Pregnant and nursing women were not enrolled. .

All subjects were in the same factory with similar education and social backgrounds. They all were requested to complete a questionnaire and had a clinical examination before sample collection. The questionnaire included a lot of information such as occupational history, personal medical history, medication used in 4 weeks before the study, body weight and height, hair dye, house decoration, radiation exposure, individual protection, smoking status and alcohol intake.

Ethical consideration

This research was approved by Medical Ethics Committee of Peking University, Health Science Center (HSC), Beijing, China. Written informed consent from each study subject was also obtained.

Air and Biological exposure assessment

According to the sampling criterion in monitoring of hazardous substances in the air (GBZ 159-2004)[23], six air sampling sections were respectively chosen in the workplaces of two groups, 10 sampling points were chosen in each section. The sampling process was used pumping at 1L/min for 8 hours (Sp730, TSI Corporation, USA), the membranes used in this study were MCE mixed cellulose ester filters (Φ37mm, pall, America). The average concentration of all sampling points on the membranes in the same group was measured by atomic absorption spectrometry[24] and then calculated to evaluate the CrA during the whole production process; the detection limit for the CrA was 0.001 μg/L.

4ml anticoagulant (EDTA and heparin) blood in peripheral was drew from each subject after finishing the questionnaire and then assigned to these two tubes on average. They were respectively used to measure the CrB and CBMN. The concentration of CrB was measured by inductively coupled plasma mass spectrometer (ICP-MS)[24]. the detection limits was 0.0012 μg/L.

At the end of the work-shift, 30mL urine sample of each subject was collected into a 50 mL metal-free polypropylene centrifuge tube (Falcon, BD Biosciences) and stored at -80℃ until used

The tubes used above had been detected their background value before research to ensure less elements and heavy metals contamination.

Determination of urinary 8-OHdG

According to the manufacturer's instructions, urine samples were firstly centrifuged at 1500 rpm for 7 min, secondly the supernatant was chosen to determine the content of urinary 8-OHdG using ELISA kit (USA Cayman chem, USA Cayman chem, 8-OHdG EIA kit) by Multiskan MK3 (Thermo, USA).The concentration of Cre in urine was determinate by alkaline picric acid assay with a commercial kit (Ausbio Laboratories Co., Ltd. China) using a Hitachi 7170A automatic analyzer (Hitachi Corp, Japan). The results of 8-OHdG were regulated by Cre to avoid the potential interference of different urine density among the subjects.

CBMN test

Peripheral venous blood which was designed in heparin tubes was taken to measure the MN frequency. The indexes (MNCC, MNC, NPB and NBUD) were counted in 1000 binuclear lymphocytes of each individual according to Fenech's protocol[25]. All scoring was carried out by two independent researchers through double - blind method. If the scoring difference from these two researchers was less than 20 percents, the average was calculated as the final result. If the scoring difference from these two researchers was more than 20 percents, another researcher should be asked to verify the scores, then the average were used after removing the most different value.

Statistical analysis

Epidata 3.0 software was used to entry the questionnaire and experimental data into computer. The whole process was utilized double-entry and logistical error check to ensure the accuracy.

All analysis was performed with SPSS16.0. Normality was assessed by Kolmogorov-Smirnov (*K-S*) test, the variables including 8-OHdG, MNCC and MNC did not meet the normality, so Log or Ln transformation was made for normality approximation. Continuous and categorical parameters between chromate exposed group and control group were tested using the two sample *t* test (or Mann-Whitney U nonparametric test) and χ^2 test. Curving correlation and linear regression were performed. Statistical significance was for two-sided. The *P* values were defined as $\alpha<0.05$.

Results

General information analysis

A total of 114 subjects including 84 chromate exposed workers (mainly in form of $K_2Cr_2O_7$) and30 controls were recruited in this study. The working age of chromate exposed group was (7.82 ± 5.51) years. Furthermore, the personal protection (gloves and masks) of workers was above 90%. The demographic characteristics of all subjects in this research were presented in Table 1, which showed that there were no significant differences in the distribution of gender, age, smoking and alcohol consumption between these two groups.

Table 1 General information of chromate exposed group and control group

Indexes	Group	Exposed group (n=84)	Control group (n=30)	*T*	χ^2	*P*
Age	$\overline{X}$±S	35.73 ±7.85	34.83 ± 8.83	0.432		0.666
≤35	n (%)	40 (47.62)	17 (56.67)		0.187	0.493
>35		44 (52.38)	13 (43.33)			
Gender	n (%)					
Male		62 (73.81)	19 (63.33)		0.869	0.351
Female		22 (26.19)	11 (36.67)			
Smoke	n (%)					
Yes		30 (35.71)	8 (26.67)		0.610	0.435
No		54 (64.29)	22 (73.33)			
Alcohol	n (%)					
Yes		29 (34.52)	16 (53.33)		2.530	0.092
No		55 (65.48)	9 (46.67)			
CrA	$\overline{X}$±S	15.45±19.00	0.23±0.38	6.963		<0.001
CrB	$\overline{X}$±S	9.45±9.47	4.05±1.87	3.215		0.018
8-OHdG (ug/g Cre)	$\overline{X}$±S	43.76±34.89	27.21±13.76	3.354		<0.001
MNCC (‰)	M(Q)	6.00 (4.00)	3.20 (2.10)	2.420		0.004
MNC (‰)	M(Q)	7.40 (4.47)	3.74 (2.94)	3.401		0.001
NBUD (‰)	M(Q)	1.11 (1.20)	1.16 (1.17)	0.163		0.871
NPB (‰)	M(Q)	1.28 (1.15)	1.42 (1.34)	0.476		0.635

Note: Smoking referred to suck at least one cigar per day and last one year or more, smoking quit but ess than one year was also included. Alcohol was definitive by weekly drinking no less than three times

Concentration of CrA and CrB

As was shown in Table 1, the concentration of CrA in chromate exposed group [(15.45±19.00) μg/m^3] was much higher than that in control group [(0.23±0.38) μg/m^3](P<0.001), but still under the exposure limitation of chromate [(50μg/m^3)(2012, ACGIH)]. The levels of CrB in chromate exposed group [(9.45± 9.47) μg/L] were also significantly higher than that in control group [(4.05±1.87) μg/L] .

Levels of urinary 8-OHdG, and serum CBMN indexes

The data distribution of indexes such as 8-OHdG, MNCC, MNC, NBUD and NPB was shown in Table 1, the levels of 8-OHdG, MNCC and MNC were all significantly higher in chromate exposed group than that in control group (P<0.05), which showed that 8-OHdG, MNCC and MNC could be used as the genetic damage biomarkers caused by chromate exposure.

Correlation

As two biomarkers for genetic damage, there was a positive correlation between log (urinary 8-OHdG) and LnMNCC or LnMNC (r=0.377 and r=0.362 respectively) P<0.05)(Figure 1).

Correlation was also analyzed between the concentration of urinary 8-OHdG and CrB. As was recorded in Figure 2 and 3, there was no linear correlation but a curve fitting between CrB and Log (8-OHdG), the value of 8-OHdG was decreased when the concentration of CrB was more than 10.50μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high exposed group (CrB ≥10.50μg/L) and the low exposed group (CrB <10.50μg/L). A positive correlation was shown between CrB and log (8-OHdG) when the CrB Level was lower than10.50μg/L (r=0.355, P<0.05), while there was a negative correlation between CrB and log (8-OHdG) when the CrB Level was higher than10.50μg/L.

The relationship between the concentration of MNCC or MNC and CrB was also analyzed in this research (Figure 4 and 5). There were no linear correlation but a

curve fitting between CrB and LnMNCC or LnMNC. The value of MNCC or MNC was decreased when the concentration of CrB was more than 9.10μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high exposed group (CrB ≥9.10μg/L) and the low exposed group (CrB <9.10μg/L). A positive correlation was shown betweenCrB and LnMNCC or LnMNC in higher chromate exposed group (r=0.365 and r=0.269 respectively, P<0.05), while a significantly negative relationship was found between CrB and LnMNCC or LnMNC in the lower chromate exposed group.(r=-0.279 and r=-0.261 respectively, P<0.05).

Discussion

Long-term and low level chromate exposure can not only increase the body's internal load but also cause a variety of harmful effects on workers' health even increasing the incidence of human cancer [26, 27]. In occupational activity, chromate could enter into workers' body mainly by respiratory system, then be metabolism and excreted by urine, so our previous researches have proved the concentration of chromate in whole blood and urine can be used as the indicators to assess chromate biological exposure[6, 28]. In this study, it was found that the concentrations of CrA and CrB in exposed group were all significantly higher than that in control group (P<0.05) ,while the CrB level in chromate exposed group was nine times more than that in the general population of our country (1.19 μg/L)[29], which showed that the conclusion was credible by contrasting the genetic damage indexes between chromate exposed group and control group.

As we all known when Cr-VI enters human blood, it will be converted into other valence chromate compounds such as Cr-III, which could not only produce a large amount of ROS that can cause the redox system imbalance, but also directly or indirectly coordinate with DNA or protein. The transformation above can affect genetic stability including oxidative DNA lesion, DNA cross-links, single and double strand breaks and so on[30,31,32]. Besides, long-term chromate exposure can also cause

cyto-toxicity to lead cells apoptosis[33].

Urinary 8-OHdG has been demonstrated as a biomarker for oxidative DNA damage in chromate exposure not only by animal experiment but also by many epidemiological researches[34, 35], because it is the site that ROS often attacks, but the dose-response relationship with occupational exposure indicators was not depicted clearly. Overall, in this research, the relationship between urinary 8-OHdG and CrB was two-way changes. The concentration of urinary 8-OHdG was not significantly increasing when the level of CrB was more than 10.50μg/L, which showed that the concentration of urinary 8-OHdG would fail to predict the degree of DNA damage when the CrB was higher than some degree. There are some reasons for this result, firstly Sumner E.R has proved that oxidative DNA damage by chromate exposure mainly targets on specifically certain glycol tic enzymes on 8-OHdG[36], which means once higher burden of CrB was produced, it could oxidize and change the structure of glycolytic enzymes to reduce the production of free 8-OHdG. secondly, some researchers have proved that 8-OHdG was not the final product of redox reaction, so when the level of CrB was higher, 8-OHdG could be converted to these further oxidation products such as Spiroiminodihydantoin (Sp)[37], so the concentration of free 8-OHdG was reduced. Thirdly, as we all know, the kidney can be damaged by chronic chromate exposure. As the level of CrB increased, the kidney was at greater health risk, which ultimately affect the exertion of 8-OHdG in urine[38].

Micronuclei is another biomarker which commonly used as the genetic damage of chromate exposure[39.40]. Many researches showed that chromate exposure could cause the increasing of MN frequency in cell research, animals experiment, and human being[41,42]. In this research, the similar conclusion was proved that the MN frequency was significantly higher in chromate exposed group than that in control group and the general population[43, 44](P<0.05). The frequencies of MNCC and MNC (as the sensitive indexes of MN) have statistical differences between these two groups. However, the dose-response relationship with CrB still need many further investigations, so this research was meaningful to discuss the suitable condition of MN as genetic damage. In this study, no linear correlation was shown between CrB

and LnMNCC or LnMNC, the MN frequency did not increase as the CrB elevated. When all subjects were divided into two subgroups by the concentration of CrB, a positive correlation was shown between CrB and LnMNCC and LnMNC in high CrB group(CrB≥9.10μg/L) (r=0.365 and r=0.269 respectively), while a significantly inverse relationship was found between CrB with LnMNCC and LnMNC in low CrB group(CrB<9.10μg/L) (r=-0.279 and r=-0.261 respectively). The reason for these results may be that the occupational chromate exposure can increase the frequency of the MN in some range. As the increasing of CrB, the cytotoxic effect might play an important role and the cells with serious genetic damage may turn into apoptosis or MN[45,46].

Both MN and urinary 8-OHdG can be used to predict different types of DNA damage in some range. A positive correlation between urinary 8-OHdG and MN was found, which suggested that these two indicators as genetic damage biomarker can be verified each other.

There was still some limitation in this study: the sample size of this study was not very large, especially the control group, so we chose some references[38,43,44] to give the value of 8-OHdG and MN in normal population to reduce the generation of bias. And it is necessary that more sample size epidemiological surveys in different chromate producing factories should be chosen to verify our conclusion.

Conclusions

All these above had provided new insights that both MN and urinary 8-OHdG can be used as the genetic damage biomarkers caused by occupational chromate exposure at some levels. The combination of these indicators can improve the credibility of the results. It is not the simple linear relationship between their concentration and the level of chromate exposure. Only when the level of CrB is below 9.10μg/L and 10.50μg/L, the MN frequency and urinary 8-OHdG can respectively show the degree of genetic damage quantitatively.

FOOTNOTES:

Contributor ship statement

All the authors included in the paper granted the criteria of the authorship. PL together with YG conceived and executed this investigation, analyzed of the data and described this manuscript. YL and JLY supported this investigation from the epidemiological aspect. GJ is responsible for the whole conduct of this study and all content of this. SFY contribute to the organization and arrangement for the scene investigation. All authors commented critically on the manuscript and agreed with this submitting.

Competing interests

There are none competing interests.

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Data sharing

No additional data available

FIGURE LEGENDS

Figure1 Correlation between LnMNCC or LnMNC with Lg (concentration of 8-OHdG in urine) in chromate exposed group

Figure 2 Correlation between urinary 8-OHdG and CrB in chromate exposed group

Figure 3 a showed the correlation between urinary 8-OHdG and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between urinary 8-OHdG and CrB in lower chromate exposed group(CrB<10.50),

Figure 4 Correlation between LnMNC or LnMNCC and CrB in chromate exposed group

Figure 5 a showed the correlation between LnMNC or LnMNCC and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between LnMNC or LnMNCC and CrB in lower chromate exposed group(CrB<10.50)

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~~A cross-sectional study: T~~the suitable condition of 8-OHdG and micronucleus as genotoxic~~—~~ ~~damage~~ biomarkers in ~~occupational~~ chromate exposed workers

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Ping Li[1], Yongen Gu*[1], Shanfa Yu[2], Yang Li[1], Jinglin Yang[1], Guang Jia[1]

* co-author: equally with the first author

1. Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

2. Department of Occupational Health Science, Institute of Occupational Medicine, Zhengzhou City, Henan Province 450052, P. R. China

Address correspondence to:

Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, P. R. China

Tel: +86-010-8280-2333, Fax: +86-010-8280-2333

E-mail: jiaguangjia@bjmu.edu.cn

Abstract

Objectives: We aimed to investigate the suitable condition of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and micronucleus (MN) as genetoxic biomarkers at different levels of occupational chromate exposure.

Design: A cross-sectional study was used.

Participants: 84 workers who exposed chromate ~~exposing chromate~~ at least one year were chosen as exposed group, while 30 non-exposed individuals were used as controls.

Main outcome measures: Environmental and biological exposure of chromate was assessed respectively by measuring the concentration of chromate in the air (CrA) and blood (CrB) by ICP-MS in all participants ~~subjects~~. CBMN ~~was conducted, in which many indexes~~ including MNCC, MNC, NPB and NBUD were calculated, while the urinary 8-OHdG was measured by ELISA method and normalized by the concentration of Cre.

Results: Compared with the control group, the levels of CrA, CrB, MNCC, MNC and 8-OHdG in chromate exposed group were all significantly higher (P<0.05). There was a positive correlation between log(8-OHdG) and LnMNCC or LnMNC (r=0.377 and r=0.362). The levels of LnMNCC , LnMNC and Log (8-OHdG) all ~~did not~~ have ~~the~~ parabola correlation ~~simple linear relationship~~ with the concentration of CrB. But ~~There~~ there was a significantly positive correlation between Log (8-OHdG) and CrB when CrB level was below 10.50 ug/L (r=0.355)~~. W~~,while a positive correlation was also found between LnMNCC or LnMNC with CrB when CrB level was lower than 9.10 ug/L(r=0.365 and r=0.269 respectively).

Conclusions: The MN and 8-OHdG can be used as the genotoxic biomarkers ~~for the genetic damage~~ in chromate exposed group, but only when CrB levels were lower

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than 9.10ug/L and 10.50ug/L respectively, they can accurately reflect the degree of genetic damage.

Key terms: ~~chromate~~Chromate; Genotoxic~~etic damage~~; 8-hydroxy-2'-deoxyguanosine~~8-OHdG~~; Micronucleus, Concentration of chromate in the blood

Abbreviation:

Cytokinesis-block micronucleus test-----CBMN

Micronuclei cell count ------MNCC

Micronucleus count -------MNC

Nuclear bridge------NPB

Nuclear bud --------NBUD

Creatinine------Cre

Strengths and limitations of this study(Article summary)

Strengths: All our results had provided new insights that only when the concentration of CrB was lower than 9.10ug/L and 10.45ug/L respectively, the MN and 8-OHdG can be used as the effective biomarkers to show the degree of genetic damage ~~for the~~ in chromate ~~occupational~~ exposed group~~ure, o~~. Otherwise, when the concentration of CrB was above these levels, the cytotoxic effects~~s~~ might play an important role and the ~~fate of~~ cells with serious genetic damage~~s~~ may turn into apoptosis or necrosis, consequently which could lead to the false appearance of lower degree of MN and 8-OHdG ~~at higher chromate exposed level~~.

Limitations: the sample size of this study ~~population~~ was not very large, especially the ~~group of~~ control ~~individuals~~group, so we chose some references to give the ~~normal~~ value of MN and 8-OHdG in normal population ~~in other controls~~ to reduce the ~~mitigation~~ generation of bias. ~~In this research, we got the recommended condition of 8-OHdG and MN as genetic damage biomarker in~~

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~~chromate exposed group.~~ ~~And It~~ it is necessary that more ~~sample size~~~~occupational~~ epidemiological surveys in different chromate producing factories should be chosen to verify ~~this~~ our conclusion.

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Introduction

Chromate is a widely used chemical in industrial and agricultural production in china, which could generate many pollutants including the waste water, ~~waste~~ gas and ~~waste~~ residue in ~~chromate~~ its production, usage, transportation and storage. Previous studies had proved that ~~Long~~long-term chromate exposure in occupational workplace ~~can~~ could affect the health status of workers even cancer, so it has been declared as a well-known environmental and occupational hazards[1].

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There are many different valences of chromate, in which the hexavalent chromate (Cr-VI) is the most harmful one. Cr-VI can enter human body mainly by inhalation during occupational activities. When entering into respiratory system (nose, bronchial and lung), some Cr-VI could accumulate in bronco-alveolar lining fluid, mucosa and pulmonary tissues[1,2] and then cross the cell membrane through non-specific phosphate/ sulfate anionic transporters to the blood. ~~be converted to Cr-V, Cr-IV and Cr-III, and accumulated in bronco-alveolar lining fluid, mucosa and pulmonary tissues~~[1,2]. ~~-~~This transformation also can consequently form many reactive intermediates (Cr-V, Cr-IV and Cr-III) and reactive oxygen species (ROS) with oxidative stress[3,4]. Both Cr-III and ROS could contribute to interact with various biological macromolecule such as Cr-DNA adducts, Cr-protein adducts, and

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protein-Cr-DNA adducts ~~proteins, DNA~~. This can ~~and other biological macromolecule to~~ cause damage on DNA and chromosome~~,~~ including base modification, single-strand breaks and double-strand breaks. These damages can result in genetic damage and ultimate carcinogenesis ~~which can initiate carcinogenesis~~ if accumulated to some degree[5, 6, 7].

Based on the evidence above, many studies have proved that ~~–~~8-OHdG can be ~~as~~used as a ~~product~~ biomarker ~~of oxidative DNA damage, can be used~~ to assess the oxidative damage and DNA mutations that are induced by the ROS in clinical[8], environmental[9,10] and occupational setting in vitro[11,12]or in cell cultures [13]. MN in peripheral blood was another biomarker to show the genetic damage. ~~in peripheral blood~~ It originates from chromosome fragments that are attacked by certain physical and chemical factors such as ~~chromium~~ chromate or whole chromosomes that lag behind at anaphase during nuclear division. When the excision-repairable DNA lesions induced in G0/G1 phase, they can be converted to MN by using inhibitors of the gap filling step of excision repairmen, so that unfilled gaps are converted to double strand breaks after S phase[14], so the frequency of MN can be used to reflect the genetic damage[15]. The CBMN test is a common method to detect the MN frequencies including many indexes such as MNCC, MNC, NPB and NBUD. In these indexes, MNCC and MNC were commonly used to detect the DNA damage[14] .

Previous studies have ~~chose~~ discussed the relationship between CrB and urinary 8-OHdG or MN in ~~to discuss the feasibility of 8-OHdG and MN as the biomarker for chromium~~ chromate ~~exposure~~exposed group to investigate the feasibility of 8-OHdG and MN as genetic damage biomarkers.~~,~~ ~~however~~However, the conclusion about this connection have yielded conflicting results: Kuo found the linear correlation between urinary 8-OHdG and CrB[16], but Gao, Kim and Zhang all didn't confirm it subsequently[17,18,19], ~~many~~ some research ~~studies~~ had been identified there was some association between CrB and MN frequencies[20,21,22], but the suitable conditions and limitations ~~for~~ of 8-OHdG and MN as genotoxic biomarkers for occupational chromate exposure have still been unclear~~.~~ So our researches aimed to observe the effect of chromate exposure on genetic damage in occupational workers, especially

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urinary 8-OHdG for the oxidative DNA damage and MN for chromosome damage. Then ~~it was~~ we discussed whether MN frequency and 8-OHdG can be used as the effective genotoxic biomarkers at different levels of chromate exposure.

Materials and methods

Study design and population

A cross-sectional survey was designed for this research. The factory was chosen as the work place in Henan province in china because (1) the product--potassium dichromate was relatively simple, most of which was water-soluble hexavalent chromate. (2) Annual health check-ups were offered in this factory for workers, which allowed us to easily collect specimens ~~from workers~~ to minimize the interference with normal work schedules.

In this research, 84 workers exposed to chromate in the factory were chosen as exposed group, while 30 non-exposed individuals working in the administration office were as control group. The criteria of subjects included: (1) workers in exposed group were at least one year employment and 3 months working in the same work position. (2) aged between 25-50. (3) no medical history of allergy, asthma or allergic rhinitis, (4) all subjects with skin infections, fever or other clinical diseases should be

excluded during the sampling period. (5) Pregnant and nursing women were not enrolled. .

All subjects were in the same factory with similar education and social backgrounds. They all were requested to complete a questionnaire and had a clinical examination before sample collection. The questionnaire included a lot of information ~~including~~ such as occupational history, personal medical history, medication used in 4 weeks before the study, body weight and height, hair dye, house decoration, radiation exposure, individual protection, smoking status and alcohol intake.

Ethical consideration

This ~~study~~ research was approved by Medical Ethics Committee of Peking University, Health Science Center (HSC), Beijing, China. Written informed consent from each study subject was also obtained.

Air and Biological exposure assessment

According to the sampling criterion in monitoring of hazardous substances in the air (GBZ 159-2004)[23], ~~Six~~ six air sampling sections were respectively chosen in the workplaces of two groups, 10 sampling points were chosen in each section. The sampling process was used pumping at 1L/min for 8 hours (Sp730, TSI Corporation, USA), the membranes used in this study were MCE mixed cellulose ester filters (Φ37mm, pall, America).The average concentration of all sampling points on the membranes in the same group was measured by atomic absorption spectrometry[24] and then calculated to evaluate the CrA during the whole production process, the detection limit for the CrA was 0.001 μg/L.

~~2ml~~ 4ml anticoagulant (EDTA and heparin) blood in peripheral was drew from each subject after finishing the questionnaire and then assigned to these two tubes on average. They were respectively used to measure the CrB and CBMN. The concentration of CrB was measured by inductively coupled plasma mass spectrometer (ICP-MS)[26][24]. the detection limits was 0.0012 μg/L.

~~The tubes used above had been detected their background value before research to ensure less elements and heavy metals contamination. .~~

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At the end of the work-shift, 30mL urine sample of each subject was collected into a 50 mL metal-free polypropylene centrifuge tube (Falcon, BD Biosciences) and stored at -80℃ until used ~~to measure the contents of 8-OHdG and Cre.~~

The tubes used above had been detected their background value before research to ensure less elements and heavy metals contamination.

Determination of urinary 8-OHdG

According to the manufacturer's instructions, urine samples were firstly centrifuged at 1500 rpm for 7 min, secondly the supernatant was chosen to determine the content of urinary 8-OHdG using ELISA kit (USA Cayman chem, USA Cayman chem, 8-OHdG EIA kit) by Multiskan MK3 (Thermo, USA) ~~and~~. The concentration of Cre in urine was determinate by alkaline picric acid assay with a commercial kit (Ausbio Laboratories Co., Ltd. China) using a Hitachi 7170A automatic analyzer (Hitachi Corp, Japan). The results of 8-OHdG were regulated by Cre to avoid the potential interference of different urine density among the subjects.

CBMN test

Peripheral venous blood which was designed in heparin ~~ized~~ tubes ~~with individual numbers~~ was taken to measure the MN frequency. The indexes (MNCC, MNC, NPB and NBUD) were counted in 1000 binuclear lymphocytes of each individual according to Fenech's protocol[23]. All scoring was carried out by two independent researchers through double - blind method. If the scoring difference from these two researchers was less than 20 percents, the average was calculated as the final result. If the scoring difference from these two researchers was more than 20 percents, another researcher should be asked to verify the scores, then the average were used after removing the most different value.

~~If the result from these two researchers was different, another researcher should be asked to verify the scores.~~

Statistical analysis

Epidata 3.0 software was used to entry the questionnaire and experimental data into computer. The whole process was utilized double-entry and logistical error check

to ensure the accuracy.

All analysis was performed with SPSS16.0. Normality was assessed by Kolmogorov-Smirnov (*K-S*) test, the variables including 8-OHdG, MNCC and MNC did not meet the normality, so Log or Ln transformation was made for normality approximation. Continuous and categorical parameters between chromate exposed group and control group were tested using the two sample *t* test (or Mann-Whitney U nonparametric test) and χ^2 test. Curving correlation and linear regression were performed. Statistical significance was for two-sided. The *P* values were defined as $\alpha<0.05$.

Results

General information analysis

A total of 114 subjects including 84 chromate exposed workers (mainly in form of $K_2Cr_2O_7$) and30 controls were recruited in this study. The working age of chromate exposed group was (7.82 ± 5.51) years. Furthermore, the personal protection (gloves and masks) of workers was above 90%. The demographic characteristics of all subjects in this research were presented in Table 1, which showed that there were no significant differences in the distribution of gender, age, smoking and alcohol consumption between these two groups.

Table 1 General information of chromate exposed group and control group

Indexes	Group	Exposed group (n=84)	Control group (n=30)	*T*	χ^2	*P*
Age	$\overline{X}$±S	35.73 ±7.85	34.83 ± 8.83	0.432		0.666
≤35	n (%)	40 (47.62)	17 (56.67)		0.187	0.493
>35		44 (52.38)	13 (43.33)			
Gender	n (%)					
Male		62 (73.81)	19 (63.33)		0.869	0.351
Female		22 (26.19)	11 (36.67)			
Smoke	n (%)					
Yes		30 (35.71)	8 (26.67)		0.610	0.435
No		54 (64.29)	22 (73.33)			
Alcohol	n (%)					
Yes		29 (34.52)	16 (53.33)		2.530	0.092
No		55 (65.48)	9 (46.67)			
CrA	$\overline{X}$±S	15.45±19.00	0.23±0.38	6.963		<0.001
CrB	$\overline{X}$±S	9.45±9.47	4.05±1.87	3.215		0.018
8-OHdG (ug/g Cre)	$\overline{X}$±S	43.76±34.89	27.21±13.76	3.354		<0.001
MNCC (‰)	M(Q)	6.00 (4.00)	3.20 (2.10)	2.420		0.004
MNC (‰)	M(Q)	7.40 (4.47)	3.74 (2.94)	3.401		0.001
NBUD (‰)	M(Q)	1.11 (1.20)	1.16 (1.17)	0.163		0.871
NPB (‰)	M(Q)	1.28 (1.15)	1.42 (1.34)	0.476		0.635

Note: Smoking referred to suck at least one cigar per day and last one year or more, smoking quit but ess than one year was also included. Alcohol was definitive by weekly drinking no less than three times

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Concentration of CrA and CrB

As was shown in ~~Figure~~Table 1, the concentration of CrA in chromate exposed group [(15.45±19.00) μg/m^3] was much higher than that in control group [(0.23±0.38) μg/m^3](*P*<0.001), but—still under the exposure limitation of chromate

[(50μg/m^3)(2012, ACGIH)]. The levels of CrB in chromate exposed group [(9.45±9.47) μg/L] were also significantly higher than that in control group [(4.05±1.87) μg/L] ~~(*P*<0.001)~~.

Levels of urinary 8-OHdG, and serum CBMN indexes

T~~As for t~~he data distribution of indexes such as 8-OHdG, MNCC, MNC, NBUD and NPB was shown in Table 1 ~~(Table 1)~~, the levels of ~~re were three kinds of indexes (~~8-OHdG, MNCC and MNC~~). They~~ were all significantly higher in chromate exposed group than that in control group (P<0.05), which showed that 8-OHdG, MNCC and MNC could be used as the genetic damage biomarkers ~~feasibly~~ caused by chromate exposure.

Correlation

As two biomarkers for genetic damage, there was a positive correlation between log (urinary 8-OHdG) and LnMNCC ~~and~~ or LnMNC (r=0.377 and r=0.362 respectively) P<0.05)(Figure ~~2~~1).

Correlation was also analyzed between the concentration of urinary 8-OHdG and CrB. As was recorded in Figure 2 and 3, there was no linear correlation but a curve fitting between CrB and Log (8-OHdG), ~~we found~~ the value of 8-OHdG was decreased when the concentration of CrB was more than 10.50μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high exposed group (CrB ≥10.50μg/L) and the low exposed group (CrB <10.50μg/L). A positive correlation was shown between CrB and log (8-OHdG) when the CrB Level was lower than10.50μg/L (r=0.355, P<0.05), while there was a negative correlation between CrB and log (8-OHdG) when the CrB Level was higher than10.50μg/L.

The relationship between the concentration of MNCC or MNC and CrB was also analyzed in this research (Figure 4 and 5). ~~there~~ There were no linear correlation but a curve fitting between CrB and LnMNCC or LnMNC. ~~We found t~~The value of MNCC or MNC was decreased when the concentration of CrB was more than 9.10μg/L. Based on the results above, the concentration of CrB was stratified into two groups: the high exposed group (CrB ≥9.10μg/L) and the low exposed group (CrB

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<9.10μg/L). A positive correlation was shown betweenCrB and LnMNCC or LnMNC in higher chromate exposed group (r=0.365 and r=0.269 respectively, P<0.05), while a significantly negative relationship was found between CrB and LnMNCC or LnMNC in the lower chromate exposed group.(r=-0.279 and r=-0.261 respectively, P<0.05).

Discussion

Long-term and low level chromate exposure can not only increase the body's internal load but also cause a variety of harmful effects on workers' health even ~~increase~~ increasing the incidence of human cancer [26, 27]. In occupational activity, chromate could enter into workers' body mainly by respiratory system, then be metabolism and excreted by urine, so our previous researches have proved the concentration of ~~chromium~~ chromate in whole blood and urine can be used as the indicators to assess chromate biological exposure[6, 28]. In this study, it was found that the concentrations of CrA and CrB in exposed group were all significantly higher than that in control group (P<0.05) ,while the CrB level in chromate exposed group was nine times more than that in the general population of our country (1.19 μg/L)[29], which showed that the conclusion was credible by contrasting the genetic damage indexes between chromate exposed group and control group.

As we all known when Cr-VI enters human blood, it will be converted into other valence chromate compounds such as Cr-III, which could not only produce a large amount of ROS that can cause the redox system imbalance, but also directly or indirectly coordinate with DNA or protein. The transformation above can affect

genetic stability including oxidative DNA lesion, DNA cross-links, single and double strand breaks and so ~~on~~[28] on[30,31,32,29,30]. Besides, long-term chromate exposure can also cause cyto-toxicity to lead cells ~~apoptosis~~[31] apoptosis[33].

Urinary 8-OHdG has been demonstrated as a biomarker for oxidative DNA damage in chromate exposure not only by animal experiment but also by many epidemiological ~~researches~~[32] researches[34,33,35], because it is the site that ROS often attacks, but the dose-response relationship with occupational exposure indicators was not depicted clearly. Overall, in this research, the relationship between urinary 8-OHdG and CrB was two-way changes. The concentration of urinary 8-OHdG was not significantly increasing when the level of CrB ~~is~~ was more than 10.50μg/L, which showed that ~~when the CrB was higher than some degree,~~ the concentration of urinary 8-OHdG would fail to predict the degree of DNA damage when the CrB was higher than some degree. There are some reasons for this result, firstly Sumner E.R has proved that oxidative DNA damage by chromate exposure mainly targets on specifically certain glycol tic enzymes on 8-~~OHdG~~[34] OHdG[36], which means once higher burden of CrB was produced, it could oxidize and change the structure of glycolytic enzymes to reduce the production of free 8-OHdG. secondly, some researchers have proved that 8-OHdG was not the final product of redox reaction, so when the level of CrB ~~is~~ was higher, 8-OHdG could be converted to these further oxidation products such as Spiroiminodihydantoin (Sp)[35,37], so the concentration of free 8-OHdG was reduced. Thirdly, as we all know, the kidney can be damaged by chronic chromate exposure. As the level of CrB increased, the kidney was at greater health risk, which ultimately affect the exertion of 8-OHdG in ~~urine~~[36] urine[38].

Micronuclei is another biomarker which commonly used as the genetic damage of chromate exposure[39,40,39,40]. Many researches showed that chromate exposure could cause the increasing of MN frequency in cell research, animals experiment, and human being[41,42,41,42]. In this research, the similar conclusion was proved that the MN frequency was significantly higher in chromate exposed group than that in control group and the general ~~population~~[41] population[43, 44] (P<0.05). The ~~frequency~~ frequencies of MNCC and MNC (as the sensitive indexes of MN) ~~has~~ have statistical

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differences between these two groups. However, the dose-response relationship with CrB still needs many further investigations, so this research was meaningful to discuss the suitable condition of MN as genetic damage. In this study, no linear correlation was shown between CrB and LnMNCC or LnMNC, the MN frequency did not increase as the CrB elevated. When all subjects were divided into two subgroups by the concentration of CrB, a positive correlation was shown between CrB and LnMNCC and LnMNC in high CrB group(CrB≥9.10μg/L) (r=0.365 and r=0.269 respectively), while a significantly inverse relationship was found between CrB with LnMNCC and LnMNC in low CrB group(CrB<9.10μg/L) (r=-0.279 and r=-0.261 respectively). The reason for these results may be that the occupational chromate exposure can increase the frequency of the MN in some range. As the increasing of CrB, more serious genotoxic damage should be caused the cytotoxic effect might play an important role and the cells with serious genetic damage may turn into apoptosis or necrosis, consequently which could lead to the false appearance of lower degree of MN such as the apoptosis or necrosis of lymphocyte, which contrarily decrease the frequency of MN in this situation[4,25,43,46].

Both MN and urinary 8-OHdG can be used to predict different types of DNA damage in some range. A positive correlation between urinary 8-OHdG and MN was found, which suggested that these two indicators as genetic damage biomarker can be verified each other.

There was still some limitation in this study: the sample size of this study was not very large, especially the control group, so we chose some references[38,43,44] to give the value of 8-OHdG and MN in normal population to reduce the generation of bias. And it is necessary that more sample size epidemiological surveys in different chromate producing factories should be chosen to verify our conclusion.

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Conclusions

All these above had provided new insights that both MN and urinary 8-OHdG can be used as the genetic damage biomarkers caused by occupational chromate exposure at some levels~~,~~ . ~~the~~ The combination of these indicators can improve the credibility of the results. It is not the simple linear relationship between their concentration and the level of chromate exposure. Only when the level of CrB is below 9.10μg/L and 10.50μg/L, the MN frequency and urinary 8-OHdG can respectively show the degree of genetic damage ~~respectively produced by chromate exposure~~ quantitatively.

Contributor ship statement

All the authors included in the paper granted the criteria of the authorship. PL together with YG conceived and executed this investigation, analyzed of the data and described this manuscript. YL and JLY supported this investigation from the epidemiological aspect. GJ is responsible for the whole conduct of this study and all content of this. SFY contribute to the organization and arrangement for the scene investigation. All authors commented critically on the manuscript and agreed with this submitting.

Competing interests

There are not competing interests.

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Data sharing

There is not data sharing.

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Figure1 Correlation between LnMNCC or LnMNC with Lg (concentration of 8-OHdG in urine) in chromate exposed group

Figure 2 Correlation between urinary 8-OHdG and CrB in chromate exposed group

Figure 3 a showed the correlation between urinary 8-OHdG and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between urinary 8-OHdG and CrB in lower chromate exposed group(CrB<10.50),

Figure 4 Correlation between LnMNC or LnMNCC and CrB in chromate exposed group

Figure 5 a showed the correlation between LnMNC or LnMNCC and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between LnMNC or LnMNCC and CrB in lower chromate exposed group(CrB<10.50)

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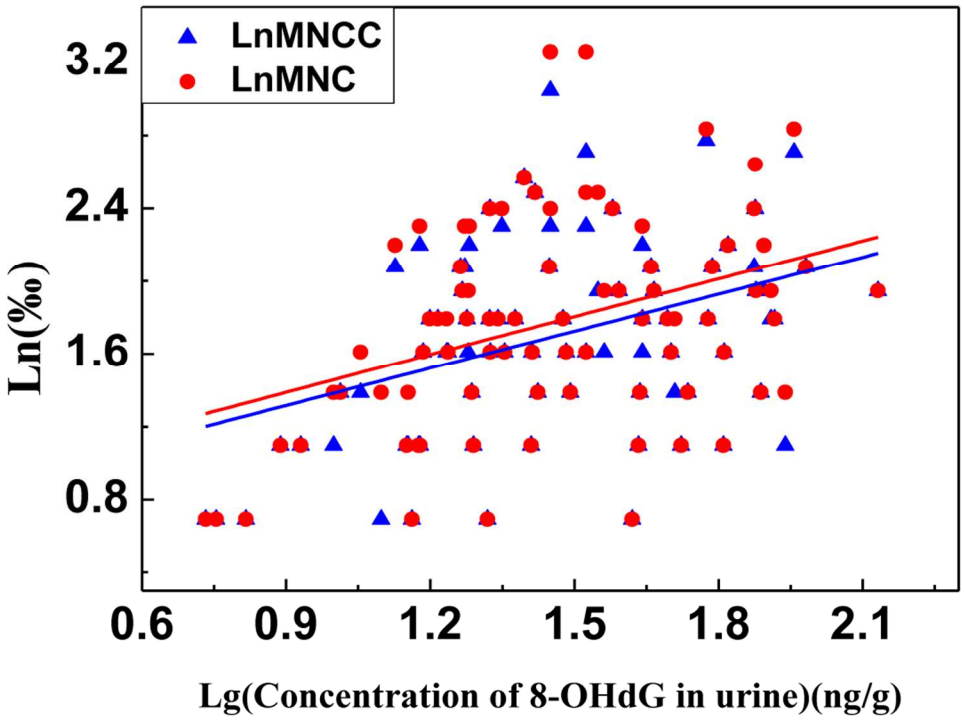

Figure1 Correlation between LnMNCC or LnMNC with Lg (concentration of 8-OHdG in urine) in chromate exposed group
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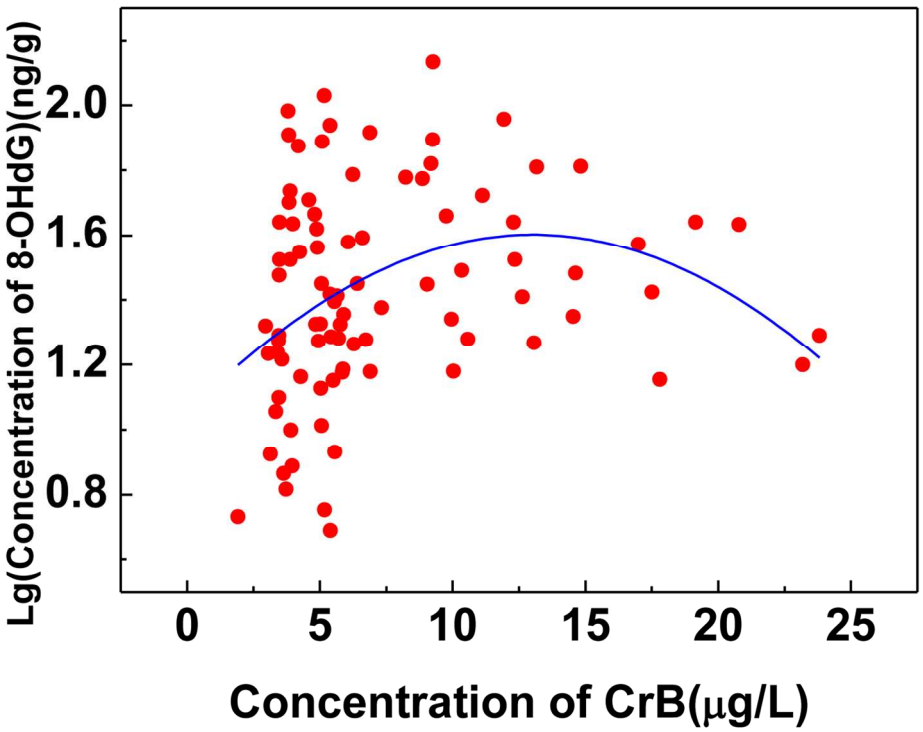

Figure 2 Correlation between urinary 8-OHdG and CrB in chromate exposed group
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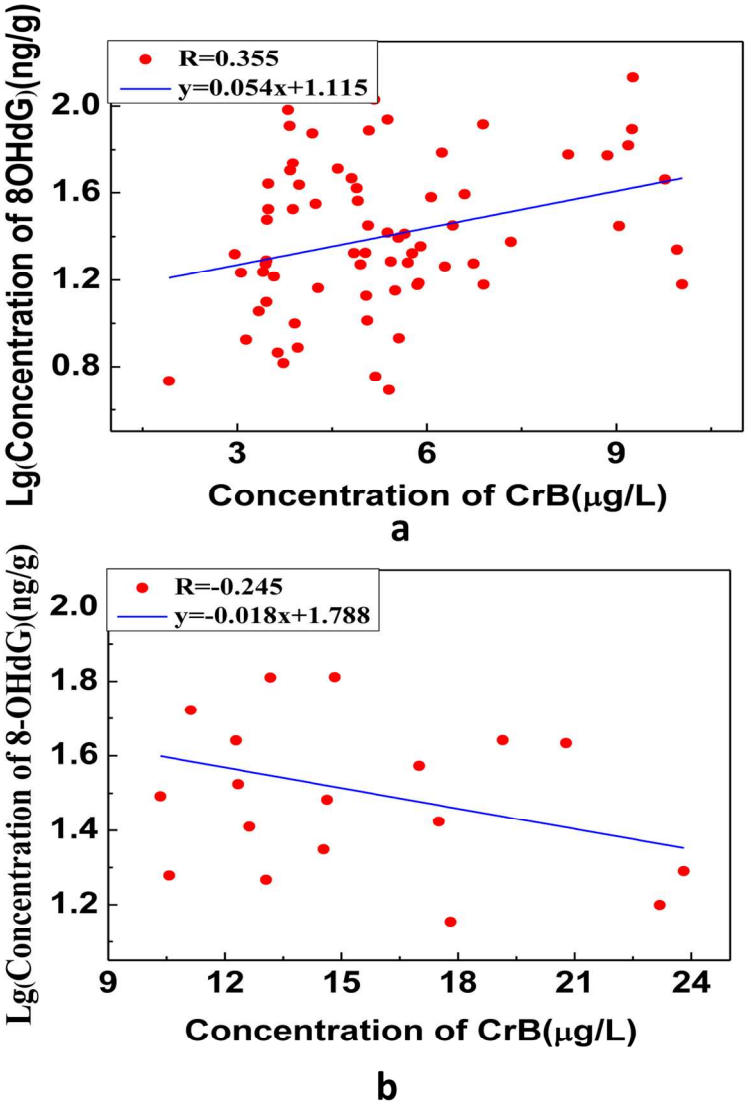

Figure 3 a showed the correlation between urinary 8-OHdG and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between urinary 8-OHdG and CrB in lower chromate exposed group(CrB<10.50)
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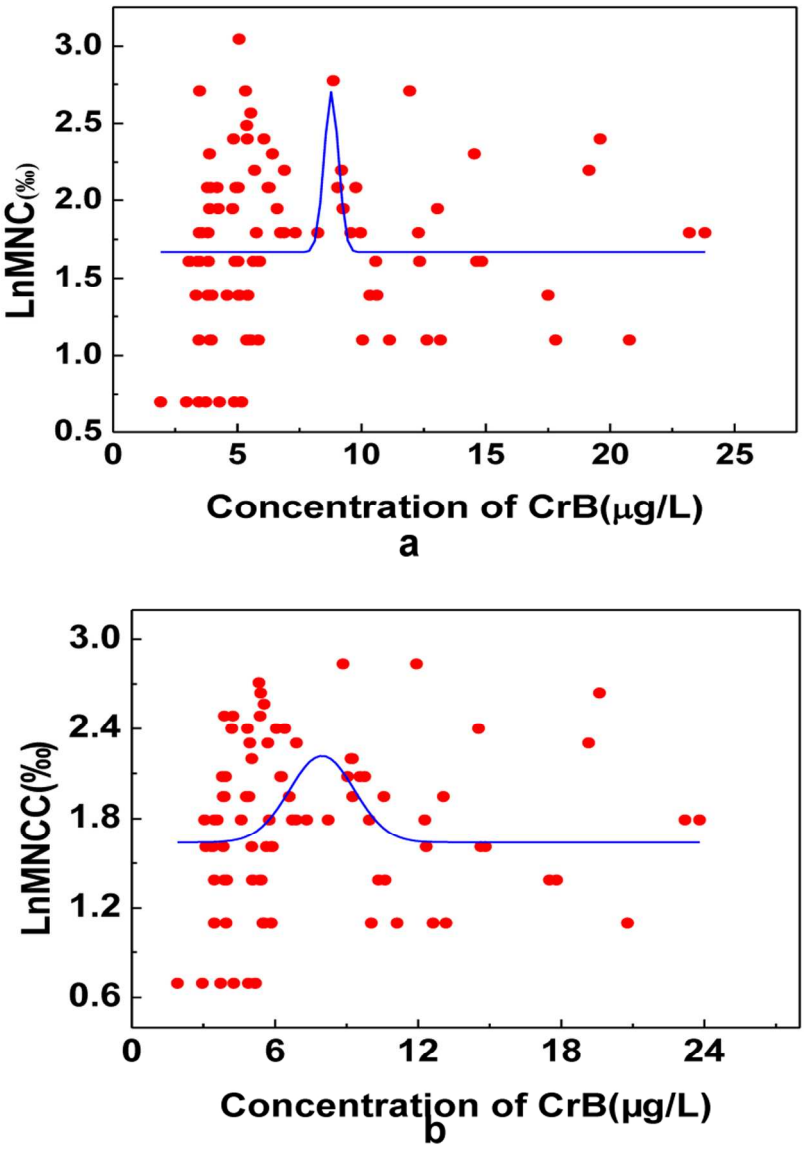

Figure 4 Correlation between LnMNC or LnMNCC and CrB in chromate exposed group
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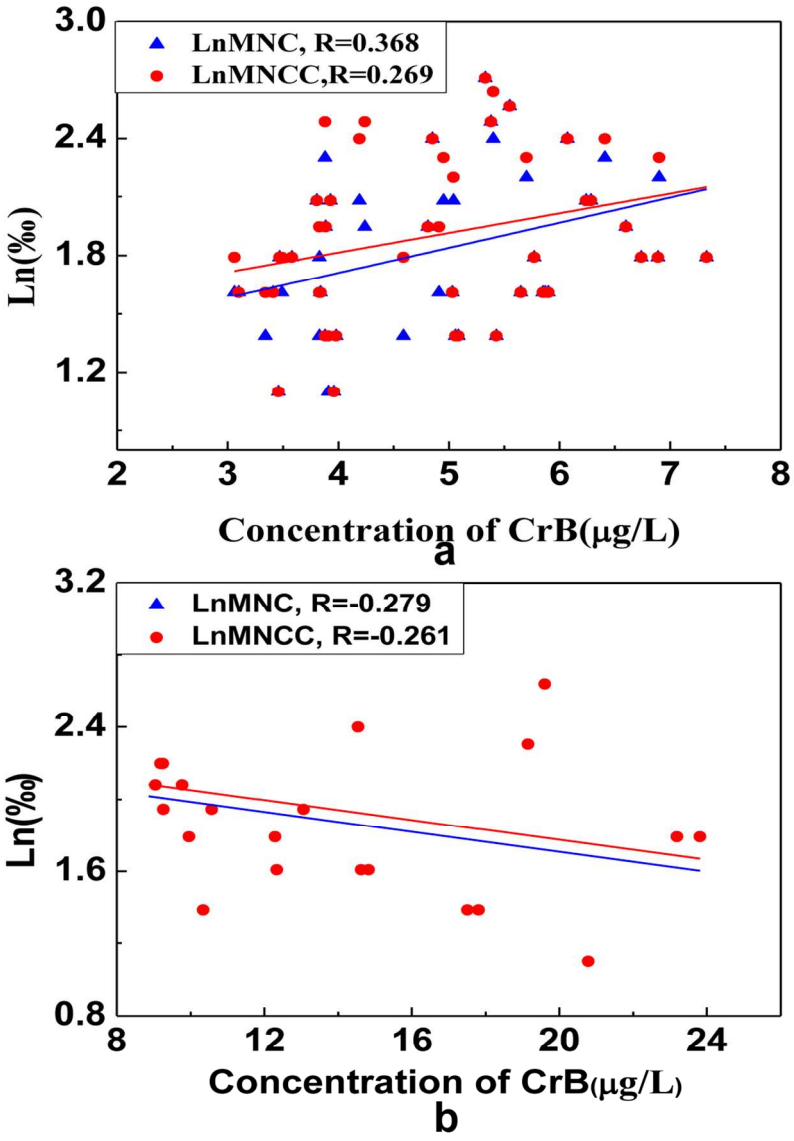

Figure 5 a showed the correlation between LnMNC or LnMNCC and CrB in higher chromate exposed group (CrB≥10.50). b showed the the linear relationship between LnMNC or LnMNCC and CrB in lower chromate exposed group(CrB<10.50)
173x230mm (300 x 300 DPI)