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Original Article

Effects of Chronic Chromate Exposure on Human Serum Prostate Specific Antigen: A Cross Sectional Study

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Abstract: The detrimental effect of chronic chromium (Cr) exposure on the prostate has never been studied. Here, we report the prostate specific antigen (PSA) changes in occupational chromate exposed workers. In this study, eighty six male occupational chromate exposed workers and forty five age-matched controls were recruited. The concentration of Cr in urine (U-Cr), serum total PSA (tPSA), free PSA (fPSA), high sensitive C reactive protein (Hs-CRP) and peripheral white blood cells count (WBC) were measured. The results show that the U-Cr, serum tPSA, Hs-CRP and WBC were significantly higher in Cr exposed workers when compared to the controls. Contrastively, the serum fPSA level in Cr exposed workers was lower than controls. A significant positive correlation between U-Cr and serum tPSA was observed. Multiple linear regression analysis revealed that serum tPSA and fPSA level was statistically associated with the serum Hs-CRP and U-Cr concentration in Cr exposed workers. These observations suggested that chronic Cr exposure could produce potential prostate injury and the nonspecific inflammation at least might be one of the reasons to explain the elevated concentration of tPSA in chronic occupational chromate exposed workers.

Key words: Chromium, Chromate, Prostate specific antigen, C reactive protein, Peripheral white blood cells count

Introduction

Chromium (Cr) compounds, widely used in industry, have been shown to have serious toxic effects on human. As a heavy metal, Cr is widespread and persistent in the environment. The increased Cr environmental pollution and occupational exposure has created a huge concern about its effect on general population and occupational workers. Previous studies have shown that exposure to Cr causes chromosome aberrations, sister chromosome exchanges, gene mutation, apoptosis and DNA oxidative damage[1)]. International Agency for Research on Cancer (IARC) already classified chromium (VI) compounds as type I carcinogen[2, 3)].

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The prostate was regarded as a critical target organ by acute exposure to Cr[4, 5] and massive exposure to Cr might result in prostate injury[6]. Several experimental studies strongly suggested that Cr can cause prostate caner in rodents[6] although epidemiological studies did not reveal the solid relationship between occupational Cr exposure and the increased risk of prostate cancer[7, 8]. Sufficient evidences showed that Cr could cause nonspecific inflammation and oxidative DNA damage *in vivo*[1, 9]. It is well-known that oxidative stress has the main triggering role in the prostate cancer development[10].

In this study, we tried to explore the relationship between occupational chromate exposure levels and the changes of serum PSA. The nonspecific inflammation was also investigated for possible mechanism of the prostate injuries.

Materials and Methods

Chromate production facility description

The studied chromate production plant was located at suburb of Jinan, Shandong province of China. The product line is consisted of chromites grinding → mixing → granulation → roasting → leaching → neutralization → acidification →concentration →separation →concentration → crystallization → centrifugation → sodium dichromate. The finished product of chromate is about 40,000 tons annually. The workers on the process of weighting or packing the end product of chromate were enrolled as chromate exposed subjects. The work place was equipped with ventilation system as required by the local government authority and the workers spent about two hours in a rest room with fresh air supply during 8 h work shift. All the workers were required to apply uniform and mask on duty. The average working time of the workers was 40 h each week.

The airborne Cr samples in the working environment were collected with FCS-30 point dust sampler (Jinqi Elect. Tech. Co. Shanghai, China) and measured with electrothermal atomic absorption spectrometric method (ET-AAS). The Cr concentrations in the air from multiple spots of the workshop were less than 50 μg/m^3. This value was below the threshold of limit value-time weighted average (TLV-TWA) as recommended both by the American Governmental Conference of Industrial Hygienists (AGCIH) and the Chinese Government.

Participants and specimen collection

The principle of the Helsinki Declaration for using human subjects was obeyed. Ethical approval for this study was granted by the Committee of the Health Science Center, Peking University and local government authorities. A written Informed Consent was obtained from all participants prior to being enrolled. All participations also completed questionnaires which mainly covered age, marital status, sex life, education, occupational career, diet as well as smoking, alcohol drinking, physical exercise, current disease, and past history of diseases. The subjects with a past/present medical history of liver diseases, renal diseases, urinary tract diseases, hypertension, diabetes and cardiovascular diseases were excluded from the study.

Eighty six male subjects who aged from 25 to 54 yr old and exposed to sodium dichromate for at least 6 months were enrolled in the study as exposed group. Forty five healthy residents (from a housekeeping company including salesman, meter checker, repairman and so on.) lived in the same city, without occupational exposure to Cr compounds or other chemicals were served as controls. The socioeconomic and demographic statuses (such as age, smoking and drinking) were matched between the chromate workers and controls as much as possible.

The post-shift urine and blood samples were collected after five consecutive work days from the Cr exposed workers. The analysis of peripheral blood WBC and urine sediments were performed within 3 h after the sample collected. The serum was obtained by centrifugation at 3,500 rpm for 10 min. Subsequently, the serum and urine samples were stored at –80°C until analysis.

Urine chromium analysis

The urinary chromium (U-Cr) was determined by ion-pair reversed-phase high performance liquid chromatograph (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) with Waters 600E HPLC system (Water Corp., USA) and X7 ICP-MS (Thermo Electron Corp., USA) as previously described[11]. The U-Cr level was adjusted with the urinary creatinine (Cre) concentration to minimize the potential interference from the sample dilution that varies among the subjects.

Measurement of the high sensitive C reactive protein (Hs-CRP) in serum

The serum Hs-CRP was measured with the Latex agglutination method with a kit purchased from Shanghai Shensuo Unf Medical Diagnostic Articles Co., Ltd (Shanghai, China) and analyzed with Olympus AU5400 automatic analyzer (Olympus Corp., Japan).

Table 1. Subject characteristics of chromate exposed workers and controls

	Chromate exposed workers			Controls			*p*
	Mean	SD	Range	Mean	SD	Range	
Age (yr)[a]	38.66	6.07	25–54	39.64	10.30	24–62	0.577
Smoker (Y/N)[b]		46/40			17/28		0.087
Drinker (Y/N)[b]		39/47			13/32		0.067
Work duration Time (yr)[a]	12.01	0.84	1–33				–
U-Cr (μg/g Cre)[c]	18.68	14.60	0.17–83.30	1.53	2.09	0.14–10.48	0.000
Serum T-PSA (ng/ml)[c]	0.75	0.55	0.17–3.00	0.51	0.20	0.21–1.26	0.046
Serum F-PSA (ng/ml)[c]	0.17	0.12	0.02–0.73	0.17	0.07	0.037–0.32	0.033
Rate of Serum F-PSA to T-PSA[c]	0.24	0.12	0.07–0.58	0.35	0.12	0.16–0.65	0.000
Serum Hs-CRP (mg/l)[c]	1.11	1.87	0.10–12.5	0.65	0.85	0.10–4.10	0.039

[a] nonparametric test (Mann-Whitney, [b] χ^2 test) or [c] student's *t*-test.

Measurement of Cre in urine

The urinary Cre was determined with the alkaline picric acid assay (Kit from Ausbio laboratories Co., Ltd., China) and analyzed with Hitachi 7170A automatic analyzer (Hitachi Corp., Japan).

Measurement of serum total prostate specific antigen (tPSA) and free prostate specific antigen level (fPSA)

The serum tPSA and fPSA level was measured by using PSA kits from Abbott Diagnostics Division (Abbott Diagnostics Corp., USA). The kits were designed based on micro-particle enzyme immunoassay (MEIA). Data were collected and analyzed with the AxSYM system (Abbott Diagnostics Corp., USA).

Blood and urine analysis

Blood was drawn from the median cubital vein into a 5ml Falcon tube containing 0.1 mM EDTA. A complete blood count (CBC) and peripheral blood cell classification were performed using the Sysmex KX-21N hematology auto analyzer (Sysmex Corp., Japan).

The urine sediment was analyzed with the Sysmex UF-100 automated sediment urinalysis (Sysmex Corp., Japan) as described by the manufacturer.

Statistical analysis

Data were processed with the statistics software of SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was tested with one sample Kolmogorov-Smirnov method. For the normal data, the student's *t*-test was used to determine the statistical significance, and the non-normality data were analyzed with the Mann-Whitney nonparametric test and spearman correlation analysis. A multiple linear regression analysis was used to verify the influence of multiple variables**,** such as age, gender, work duration time, U-Cr, serum Hs-CRP concentration and peripheral blood parameters, on serum PSA concentration in Cr exposed workers. The serum tPSA or fPSA concentration was treated as dependent variables, while age, gender, duration time, U-Cr, serum Hs-CRP concentration and peripheral blood parameters as independent values. The U-Cr data did not follow the normal distribution model and were transformed into natural logarithm before they were analyzed with multiple regression method. All reported *p* values were two-tailed and the *p* values of less than 0.05 were considered to be statistical significance.

Results

The general characteristics of Cr exposed workers and controls were listed in Table 1. There were no significant difference in age distribution, smoking and drinking habit between Cr exposed workers and control group.

Cr workers in this study had been exposed to airborne Cr of a relatively constant concentration in the workplace for at least six months. So we used U-Cr instead of Cr in RBC, which is more complex to detect, as a biomarker of chronic exposure. The U-Cr concentration of both Cr exposed workers and controls was shown in Table 1, the mean of U-Cr levels in Cr exposure group was significantly higher than the control group ($p<0.01$).

The inflammatory response factor, serum Hs-CRP concentration was listed in Table 1. The serum Hs-CRP in Cr exposed workers was elevated significantly comparing to the controls.

In the Cr exposed workers, the serum tPSA was significantly higher than that of controls. Contrastively, the serum fPSA level in Cr exposed workers was significantly

Table 2. Peripheral blood WBC count and classification of the chromate exposed workers and controls

	Chromate exposed workers			Controls			*p*
	Mean	SD	Range	Mean	SD	Range	
WBC (10^9/l)	6.96	1.72	3.7–13.5	6.17	1.32	4.0–9.7	0.025
LYM (%)	32.58	8.32	12.6–53.8	33.86	4.75	21.3–45.6	0.528
MXD (%)	8.49	3.32	2.7–20.0	7.14	7.13	4.7–12.2	0.003
NEUT (%)	58.93	9.75	33.4–82.1	59.00	5.34	44.7–71.8	0.957
LYM (10^9/l)	2.23	0.67	0.8–4.8	2.07	0.47	1.3–3.7	0.193
MXD (10^9/l)	0.59	0.26	0.2–1.4	0.44	0.13	0.2–0.9	0.000
NEUT (10^9/l)	4.14	1.43	1.5–9.8	3.66	0.96	1.8–6.6	0.057

Nonparametric Test (Mann-Whitney test). NEUT (Neutrophils), LYM (Lymphocytes), MXD (Mixture WBC which including the monocytes, eosinophils and basophils).

Table 3. Urinary sediment examination of chromate exposed workers and controls

	Chromate exposed workers			Controls			*p*
	Mean	SD	Range	Mean	SD	Range	
RBC (/μl)	5.28	9.91	0.10–74.00	3.80	2.98	0.30–11.80	0.180
WBC (/μl)	5.74	11.73	0.00–88.10	3.72	4.40	0.30–20.70	0.797
EC (/μl)	2.69	8.56	0.00–74.30	0.85	0.76	0.00–3.10	0.014
Cast (/μl)	0.32	1.83	0.00–16.99	0.09	0.15	0.00–0.77	0.967
Bact (/μl)	1,314.27	1,208.02	89–7,496.80	1,212.30	677.70	193.50–2,834.80	0.541
Path Cast (/μl)	0.08	0.54	0.00–5.00	0.03	0.09	0.00–0.40	0.785
SRC (/μl)	0.56	0.62	0.00–4.40	0.28	0.27	0.00–1.10	0.001
X'TAL (/μl)	56.18	151.93	0.60–1,118.90	23.47	57.70	0.70–274.10	0.056
YLC(/μl)	0.00	0.00	–	0.00	0.00	–	–
Non-lysed RBC (/μl)	4.15	7.09	0.00–45.40	3.06	2.50	0.3–9.80	0.363
Non-lysed RBC %	80.26	19.98	15.70–100.0	78.17	17.72	28.00–100.0	0.310

Nonparametric Test (Mann-Whitney Test).

lower than the controls (Table 1). We further calculated the ratio of fPSA to tPSA to figure out the intrinsic correlation between Cr exposure and prostate injury. The fPSA/tPSA was significantly lower in Cr exposed workers than in the controls.

The data of the peripheral white blood cell (WBC) count and classification in Cr exposed workers and controls were shown in Table 2. The peripheral total WBC count in the Cr exposed workers was significantly higher than that of controls (p=0.025). The difference of peripheral WBC count showed that the number and percentage of the mixture WBC (MXD) (which including monocytes, eosinophils and basophils) increased significantly, while the number and percentage of neutrophils (NEUT) and lymphocytes (LYM) showed no significant difference between Cr exposed workers and controls.

The urinary sediment analysis showed that the number of the urinary squamous epithelial cells (EC) and small round cells (SRC) in Cr exposed workers were significantly higher than controls (Table 3). There was no significant difference as to the urine RBC and WBC between Cr exposed workers and controls (Table 3).

In Cr exposed workers, the U-Cr level was significantly correlated with the serum tPSA level (r=0.232, p=0.036, Fig. 1). A significantly positive correlation between peripheral blood WBC count and serum Hs-CRP concentration was also observed (r=0.348, p=0.001, Fig. 2). The multiple linear regression analysis revealed that serum tPSA and fPSA level was statistically associated with the serum Hs-CRP and U-Cr concentration in Cr exposed workers (Table 4).

Discussion

Exposure to Cr may induce epithelial proliferation and oxidative DNA damage in target organs which might con-

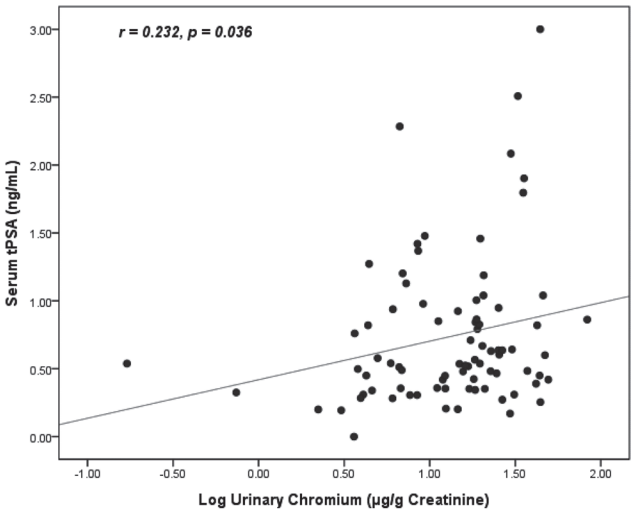

Fig. 1. The correlation between serum tPSA and U-Cr concentration in occupational chromate exposed workers.
The serum tPSA and U-Cr were measured as described in the text. The correlation between tPSA and U-Cr was calculated using multiple linear regression analysis.

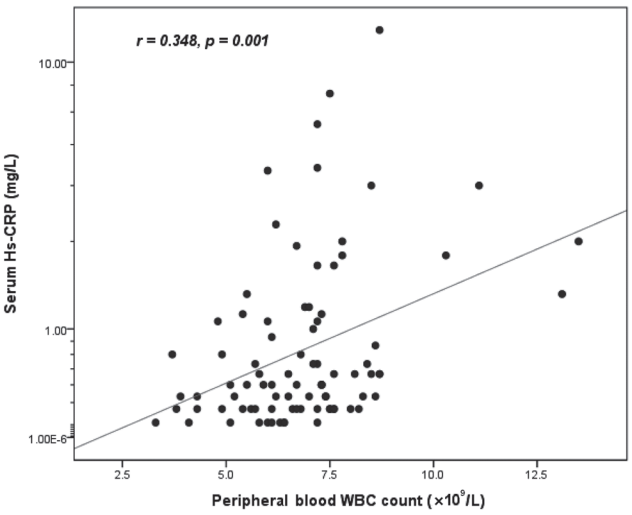

Fig. 2. The correlation between peripheral blood WBC and serum Hs-CRP concentration in occupational chromate exposed workers.
All subjects were submitted for WBC count analysis and Hs-CPR measurement after consented participation in the study. The correlation between WBC and Hs-CRP was calculated using multiple linear regression analysis.

Table 4. Multiple linear regression analysis of serum t-PSA or f-PSA in occupational chromate exposed workers

	Variable	B	Std error	β	*t*	*p*
Serum tPSA	Constant	0.430	0.447	–	0.97	0.338
	Age	–0.005	0.010	–0.005	–0.013	0.610
	Duration Time	0.000	0.010	0.001	0.012	0.991
	Peripheral blood WBC Count	–0.001	0.036	–0.003	–0.027	0.978
	Serum Hs-CRP	0.09	0.037	0.28	2.38	0.020
	Log U-Cr	0.37	0.14	0.29	2.50	0.012
Serum fPSA	Constant	0.15	0.10	–	1.49	0.139
	Age	–0.001	0.002	–0.04	–0.33	0.732
	Duration Time	0.001	0.002	0.03	0.27	0.790
	Peripheral blood WBC Count	–0.009	0.008	–0.13	–1.008	0.280
	Serum Hs-CRP	0.018	0.008	0.26	2.16	0.034
	Log U-Cr	0.067	0.03	0.23	2.035	0.045

Multiple regressions: enter method. The B was the unstandardized coefficients and the β was the standardized coefficients.

tribute to the carcinogenesis[1, 9, 15]. The prostate could be one of the target organs for occupational and environmental Cr exposure in addition to other well reported organs, such as the lung and kidney. However, there is very limit information on the adverse effect of Cr on the prostate, particularly for chronic exposure.

PSA is a glycoprotein that is expressed by both normal and neoplastic prostate tissue[12]. In the normal physical condition, very low level of PSA in blood can be detected. It is believed that the elevated serum PSA concentration is always associated with certain pathological changes in the prostate including malignant metaplasia, various benign prostatic hyperplasia (BPH), prostatic manipulation, asymptomatic and chronic prostatitis[13, 14] and elevated serum PSA usually indicates prostatic epithelial damage[13, 16]. Our study showed that the serum tPSA in Cr exposed workers was significantly higher than the controls and positively associated with U-Cr. The above results suggested that

the increased serum tPSA could predict the prostate injury produced by chromate exposure.

Under healthy condition, the serum fPSA represents the matured PSA protein which has been inactivated by internal proteolytic cleavage. This cleaved fraction is dropped in patients with prostate cancer[17]. Thus, the lowered fPSA (free or unbound PSA) in the serum is a supporting indicator for prostate cancer, while the amount of PSA (complex PSA) is higher compared with those who have normal prostates or BPH[18–20]. It is therefore, the ratio of fPSA to tPSA has been used as a diagnostic indicator to distinguish prostate cancer and BPH clinically. In this study, with the increased serum tPSA concentration in Cr exposed worker, the serum fPSA level and the ratio of serum fPSA to tPSA decreased as compared to the controls. The World Health Organization (WHO) has declared that chromium (VI) compounds are typical human carcinogen. Meanwhile both the Department of Health and Human Services (DHHS) and Environmental Protection Agency in the United States have also announced that ambient chromium (VI) is a human carcinogen. Due to no cut-point value existed for PSA to predict prostate cancer with high sensitivity and high specificity in clinical medicine[21, 22], our data can only ring the bell for attention on the chronic occupational Cr exposed population with potential risk of prostate cancer.

Prostate cancer and BPH are both often accompanied with inflammation[23–25] which can cause serum PSA elevation[26]. The peripheral blood WBC count and classification analysis may indicate the existence of the inflammation[27], particularly the neutrophils often indicate the presence of an acute infection. In our study, the peripheral blood WBC count was significantly elevated in occupational Cr exposed workers and the classification analysis of WBC showed the increased proportion of mononuclear cells rather than the neutrophils, which supported that nonspecific inflammatory response might be involved in the effects of Cr on the prostate.

On the other hand, C reactive protein (CRP) is a positive acute phase protein synthesized by the liver in response to inflammation. It serves as a nonspecific marker of inflammation and may reflect the presence of inflammation in the body. The increased serum Hs-CRP level has been reported in the acute or chronic prostate inflammation, BPH and prostate cancer[28, 29] although it is non-specific. The CRP has also been used as a prognostic marker for prostatic cancer[30, 31]. In this study, the serum Hs-CRP level of Cr exposed workers was significantly higher than the controls. The elevated serum Hs-CRP level showed positive correlation with blood WBC count and the multiple linear regression analysis demonstrated that the concentration of serum tPSA and fPSA level were significantly associated with serum Hs-CRP and U-Cr level in Cr exposed workers. These data further indicated that the occupational Cr exposure could produce prostate cellular injury and possible cancerous development. Well, further investigation with better elaborated technical assays is required to confirm this presumption.

The BPH is a common condition afflicting elder population and is often accompanied with urinary tract infection. Urinary sediment examination is a useful indicator of pathological alterations in the urinary system. Elevated urinary WBC may indicate bacterial infection or inflammation. Epithelial cells in urine may come from the kidney, urinary tract and prostate. And the increased numbers of epithelial cells in urinary sediment are associated with irritation and neoplastic transformation[14, 32]. Our analysis showed that the epithelial cells (ECs) and small round cells (SRCs) were significantly increased in Cr exposed workers. The above results demonstrated that there was no significant infective inflammation in the urinary system from the Cr exposed workers. The increased ECs and SRCs might be due to the irritation or injury caused by Cr.

Both peripheral blood WBC and serum HS-CRP were considered as acute effect index. As we know, the changes of peripheral blood WBC count and classification as well as serum hs-CRP concentration in human body can be influenced by a lot of different factors such as the status of body health, work stress, smoking, drinking, eating habits, and sometimes even family income except the chromate exposure or infection. Here, to control the confounding factors efficiently, clinical screening is employed for removal of the effects of infections and systemic diseases in both exposed and control groups. Furthermore, both of the groups were matched in view of respective factors such as age, gender, smoking, drinking and other life styles. Nonetheless, in order to confirm the causality among serum PSA, peripheral blood WBC count and classification as well as serum hs-CRP concentration in chromate exposed workers, a well designed prospective cohort study needs to be performed.

In conclusion, the serum tPSA was increased and a nonspecific inflammation was existed in occupational chromate exposed workers. The higher level of tPSA accompanied with the lowered ratio of tPSA to fPSA warranted health monitoring on prostate in chromate exposed workers.

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