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Exposure estimates of chromeplaters in India: an exploratory study

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Abstract
The literature has a paucity of knowledge on the exposure and effect estimates of chromeplaters in India. In an exploratory endeavour on chromium (Cr) exposure risk assessment, blood and urinary Cr levels plus the DNA–protein crosslink content were analysed in peripheral blood lymphocytes of chromeplaters ($n=24$). A cross-sectional study design was selected. Non-chromeplaters ($n=35$) were taken as the matching control. The results show that levels of blood and urinary Cr were greater in chromeplaters. A significant increase in DNA–protein crosslink coefficients of peripheral blood lymphocytes and urinary Cr levels was observed. The results demonstrate higher exposure estimates in chromeplaters and reveal exposure to a biologically effective dose of the toxic metal. The study also validated the employed biomarkers for Cr exposure risk assessment.

Keywords: *Biomarker, urinary and blood chromium levels, DNA–protein crosslinks, lymphocytes*

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Introduction

Hexavalent chromium (Cr^{6+}) compounds are occupational carcinogens (International Agency for Research on Cancer 1990). Chromic acid mist (a Cr^{6+} compound) is generated at chromeplating workstations raising the exposure risk of workers to the carcinogen. A substantial population of chromeplaters thus is exposed to Cr chronically and consequently faces a risk of Cr-specific health hazards. Dermatitis, ulcer formation, nasal septum perforation, inflammation, and carcinoma of the larynx and lung parenchyma and paranasal sinuses, etc. are the health hazards of exposure to Cr (Walsh 1953, Browning 1969, Royle 1975, Langardt 1990). According to a conservative estimate, approximately 1 million subjects are engaged in chromeplating occupation in India. However, their exposure and health effect estimates are rarely reported (Danadevi et al. 2004).

In the literature, the analyses of urinary/blood Cr levels and the content of DNA–protein crosslinks (DPCs) in peripheral blood lymphocytes (PBL) have been employed as Cr exposure markers (Coogan et al. 1991, Gao et al. 1993, Zhitkovich et al. 1998). In blood, both erythrocytes and lymphocytes are capable of accumulating Cr. Nevertheless, plasma levels of Cr are also reported (Angerer et al. 1987). This is

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for the reason of Cr^{6+} metabolism in blood cells as well as in plasma (Korrallus et al. 1984). The Cr levels in lymphocytes exclusively does not serve as a good exposure marker. Unlike red blood cells, these lack the active transport characteristics (Lukanova et al. 1996). Lymphocytes are nucleated cells and can accumulate Cr. Hence, PBL serve as a surrogate tissue to study cumulative DNA damage, i.e. DPC formation. Cellular DPCs are biologically active nucleoprotein complexes and the validated biomarker for Cr exposure assessments. DPCs are induced following exposure to only a select number of carcinogens, i.e. ultraviolet light radiation, formaldehyde, nickel, Cr, alkylating agents and platinum compounds. Induction is described both in experimental systems as well as in humans (Tsapakos et al. 1983, Wedrychowski et al. 1985, Langardt 1990, Taioli et al. 1995). Cr compounds, of both 3^+ and 6^+ oxidation states, induce DPC formation in human blood cells (Medeiros et al. 2003).

The present study reports urinary/blood Cr levels and the contents of DPC in PBL. Cr levels in urine and blood samples imply the internal exposure estimates. DPC contents in PBL denote the biological-effect estimates. The study was conducted in a chromeplating unit in an industry where adequate environmental safety arrangements were already in place. The work environment was well ventilated and equipped with proper exhaust fans.

Materials and methods

Histopaque-1077, 3,4-diamino benzoic acid, Standard Reference Material for Cr and proteinase K were obtained from M/S Sigma Aldrich (Bangalore, India). The other chemicals were of analytical grade and procured from Merck, Qualigens, Sarabhai Chemicals, India.

Study population and design

A small group of chromeplaters ($n = 24$) and the suitable control (non-chromeplaters, $n = 35$) participated in the study. The control group consisted of subjects of similar age, sex, body mass index and socio-economic status, but who did not work in the chromeplating units in the recent past. Occasional exposure to the trivalent form of Cr in the distant past was, however, recorded in sporadic incidences. Informed consent was obtained from each person. A cross-sectional study design was used. A medical doctor examined the study population for clinical abnormalities using a structured questionnaire, which included the demographic details on their current health status, work-related health complaints, smoking/alcohol consumption history, their present and past occupational history of exposure to Cr^{6+} compounds, etc. Consumption of vitamins or antioxidants in the immediate past was also recorded.

Venous blood samples were collected in heparinized tubes and spot urine samples in sterile plastic containers. Samples were transported to the laboratory under chilled conditions. Lymphocytes were isolated on Histopaque-1077 within 3–5 h after collection.

Occupational exposure zones and exposure situations

The chromeplating work was conducted manually in a rectangular large room (17 length $\times$ 17 width $\times$ 10 m height). It was at an isolated location and distant from other

units in the studied industry. Chromeplating workers remained in the same hall during working hours irrespective of their involvement in the activity. This was usually due to adverse climatic conditions such as extreme cold/hot/rainy weather as well as to occasional staying-in offhandedly during recess periods. While chromeplating, the workers put objects into the bath manually and took them out just merely avoiding touching the chromeplating solution in the bath.

Cr analysis

Urinary and blood Cr analysis was done by a direct dilution method. An atomic absorption spectrophotometer equipped with an HGA 400 graphite furnace (Perkin-Elmer model 4000) was used for Cr estimations. The samples were diluted with 0.1% Triton X-100 and 1% HNO_3 (Schermaier et al. 1985) and analysed directly. Samples were spiked occasionally for per cent recovery analysis, which was 80%. The results were corrected accordingly. A solution of Cr standard reference material (1000 ppm as chromic nitrate) was used for calibration and analysis after dilution to 5–20 ppb.

DNA–protein crosslink assay

Lymphocytes (about 1 million) were de-proteinized in 1% SDS-10 mM Tris-HCl pH 7.5 and stored frozen at -20°C until analysis. DPC were extracted using SDS and potassium salt (Browning 1969, Zhitkovich & Costa 1992). Protein-bound DNA was measured fluorimetrically after protein digestion with proteinase K and the values expressed as DPC content and DPC coefficient. DPC content was expressed as the SDS-precipitable (protein-bound) DNA per million cells; the DPC coefficient as the ratio of SDS-precipitable (protein-bound) DNA to total DNA (protein free).

Statistical analysis

Statistical analysis was done using non-parametric Mann–Whitney rank sum test to evaluate the significance of change in different parameters in the control and exposed populations.

Results

The mean age of chromeplaters ($n=24$) was 44.29 ± 8.16 years (range 25–56 years) and that of the non-chromeplaters ($n=35$) was 32.28 ± 9.97 years (range 19–70 years). The mean duration of exposure to Cr in chromeplaters was 14.54 ± 8.59 years (range 3–27 years). The same in non-chromeplaters was 1.16 ± 0.37 years (range 1–2 years) due to occasional working with the trivalent form of Cr in the distant past. A substantial population of chromeplaters (30%) were smokers as against the insignificant number (3%) of non-chromeplaters. No correlation was found between smoking and exposure estimates.

Among chromeplaters, approximately 40% ($n=8$) of the population revealed clinical symptoms. A group of six subjects (25%) reported redness in conjunctiva with prominent bulbar vessels. Two subjects (8.3%) reported congestion of nasal mucosa. Nearly 10% ($n=3$) of chromeplaters reported dermatological ailments such as rashes and papule along with complaints of itching/burning sensation. None of the studied population, however, gave a history of a secondary job with a possibility of exposure to

Cr or antioxidant therapy in the immediate past. The workload of the chromeplaters was comparatively light. No clinical symptoms were found in non-chromeplating subjects.

The results of urinary/blood Cr analyses and levels of DPC and DPC coefficients are summarized in Table I. The chromeplating subjects revealed higher mean urinary Cr level than the non-chromeplaters. The change was significant ($p = 0.001$). The range of urinary Cr levels in chromeplaters was also greater compared with the corresponding control. Mean blood Cr levels in the chromeplaters showed a similar trend. However, the change was non-significant ($p < 0.103$).

The mean DPC content and coefficients were greater in the Cr-exposed population than in the corresponding control. The range of values also showed a similar pattern in both parameters. The change in DPC coefficients was highly significant.

Discussion

The results are comparable with those reported in the literature (Imbus et al. 1963, Angerer et al. 1987, Pierce & Cholak 1996, Merzenich et al. 2001).

The blood/urinary Cr levels provided the internal exposure estimates of Indian chromeplaters. The marker levels were greater in Cr handlers. Higher exposure estimates confirmed exposure to Cr. The marker levels represented the total exposure to Cr including the dietary, dermal and inhalation routes of exposure.

A higher concentration of the exposure marker DPC in chromeplaters was evident from the analysis of the DPC coefficient. The data suggested exposure of workers to biologically effective doses of Cr. This marker is specific to Cr exposure and could be a function of Cr-induced oxidative stress (Taioli et al. 1995). The physiological significance of this type of DNA damage in Cr toxicity is not well understood. However, the biochemical change may be linked to the loss of genetic information during DNA replication and transcription, which may inactivate certain genes and

Table I. Chromium exposure and effect estimates.

Parameters	Non-chromeplaters	Chromeplaters	Mann–Whitney *U*-test (p)
Urinary Cr (ppb):			
Mean ± SD (n)	1.83 ± 0.66 ($n = 11$)	4.85 ± 2.34* ($n = 18$)	
Range	0.8–3.08	1.46–9.84	
Rank sum	88	318	22.00* ($p = 0.001$)
Blood Cr (ppb):			
Mean ± SD (n)	1.93 ± 0.34 ($n = 24$)	2.32 ± 0.70 ($n = 15$)	
Range	0.9–2.48	1.26–3.67	
Rank sum	423.5	356.5	123.5 ($p = 0.103$)
DPC content (μg DNA/million cells):			
Mean ± SD (n)	0.88 ± 0.85 ($n = 25$)	1.95 ± 1.82 ($n = 17$)	
Range	0.05–3	0.02–8.33	
Rank sum	527	419	149 ($p = 0.092$)
DPC coefficient:			
Mean ± SD (n)	0.03 ± 0.01 ($n = 25$)	0.10 ± 0.08* ($n = 17$)	
Range	0.001–0.125	0.02–0.36	
Rank sum	362	458	62* ($p < 0.001$)

*Significant.

upset their expression pattern otherwise vital for cell differentiation and growth (Zhitkovich et al. 1992). In human studies, DPC values are not affected by weight, age, race, gender or smoking among both control and exposed population (Popp et al. 1991, Costa et al. 1993, Taioli et al. 1995).

The clinical observations in the studied subjects showed a weak association with the respective exposure estimates. A weak association could possibly be due to small sample size or the result of other confounding factors, e.g. less workload and occupational environment levels of Cr, life style and living conditions. In our study, smoking was not a contributing factor for the higher exposure estimates in chromeplaters.

Similar studies with a relatively large sample size are needed to confirm these observations. Further investigations are also needed to evaluate the role of other contributory factors (e.g. ambient air Cr levels, dietary habits and improper nutrition) in clinical presentations of chromeplaters especially against the backdrop of their social, ethnic or economic diversity.

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References

Angerer J, Amin W, Heinrich-Ramm R, Szadkowski D, Lehnert G. 1987. Occupational chronic exposure to metals: chromium exposure of stainless steel welders — biological monitoring. International Archives of Occupational and Environmental Health 59:503–512.

Browning E. 1969. Toxicity of industrial metals. 2nd Edn. London: Butterworth. p. 119–131, 249–260.

Coogan T, Squibb K, Motz J, Kinney P, Costa M. 1991. Distribution of chromium within cells of the blood. Toxicology and Applied Pharmacology 108:157–166.

Costa M, Zhitkovich A, Toniolo P. 1993. DNA protein crosslinks in welders: molecular implication. Cancer Research 53:460–463.

Danadevi K, Rozati R, Banu BS, Grover P. 2004. Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the comet and micronucleus assays. Mutagenesis 19:35–41.

Gao M, Levy LS, Braithwaite RA, Brown SS. 1993. Monitoring of total chromium in rat fluids and lymphocytes following intratracheal administration of soluble trivalent and hexavalent chromium compounds. Human Experimental Toxicology 12:377–382.

International Agency for Research on Cancer. 1990. Chromium, nickel and welding. Monographs on the Carcinogenic Risk to Humans, Vol. 49. Lyon: IARC.

Imbus HR, Cholak J, Miller LH, Sterling T. 1963. Boron, cadmium, chromium and nickel in blood and urine. A survey of American working men. Archives on Environmental Health 6:286–295.

Korrallus U, Harzdorf C, Lewalter J. 1984. Experimental bases for ascorbic acid therapy of poisoning by hexavalent chromium compounds. International Archives of Occupational and Environmental Health 53:247–256.

Langardt S. 1990. One hundred years of chromium and cancer: a review of epidemiologcal evidence and selected case reports. American Journal of Industrial Medicine 17:189–215.

Lukanova A, Toniolo P, Zhitkovich A, Nikolova V, Panev T, Popov T, Taioli. E, Costa M. 1996. Occupational exposure to exposure to Cr (VI): comparison between chromium levels in lymphocytes, erythrocytes and urine. International Archives of Occupational and Environmental Health 69:39–44.

Medeiros MG, Rodrigues AS, Batoreu MC, Laires, Reuff A, Zhitcovich A. 2003. Elevated levels of DNA–protein crosslinks and micronuclei in peripheral lymphocyte of tannery workers exposed to trivalent chromium. Mutagenesis 18:19–24.

Merzenich H, Hartwig A, Ahrens W, Beyersmann D, Schlepegrell R, Scholze M, Timm J, Jockel KH. 2001. Biomonitoring on carcinogenic metals and oxidative DNA damage in a cross-sectional study. Cancer Epidemiology Biomarkers and Prevention 10:515–522.

Pierce JO, Cholak J. 1966. Lead, chromium and molybdenum by atomic absorbtion. Archives on Environmental Health 13:208–212.

Popp W, Vahrenholz C, Schmielding. W, Krewet E, Norpoth K. 1991. Investigations of the frequency of the DNA strand breakage and crosslinking and of sister chromatid exchange in the lymphocytes of electric welders exposed to chromium and nickel containing fumes. International Archives of Occupational and Environmental Health 63:115–120.

Royle H. 1975. Toxicity of chromic acid in chromium plating industry (i). Environmental Research 10:39–53.

Schermaier AJ, O'Connor LH, Pearson KH. 1985. Semi-automated determination of chromium in whole blood and serum by zeeman electrothermal atomic absorbtion spectrophotometry. Clinica et Chimica Acta 152:123–134.

Taioli E, Zhitkovich A, Kinney P, Udesin K, Toniolo P, Costa M. 1995. Increased DNA–protein crosslinks in lymphocytes of residents living in Cr contaminated areas. Biology and Trace Elements Research 50:175–180.

Tsapakos MJ, Hampton TH, Wetterhahn KE. 1983. Chromium(VI) induced DNA lesions and Cr distribution in rat kidney, liver and lung. Cancer Research 43:5662–5667.

Walsh EN. 1953. Chromate hazards in industry. Journal of the American Medical Association 153:1305–1308.

Wedrychowski A, Ward WS, Schmidt WN, Hnilica LS. 1985. Chromium induced crosslinking of nuclear protein 8 and DNA. Journal of Biological Chemistry 260:7150–7155.

Zhitkovich A, Costa M. 1992. A simple, sensitive assay to detect DNA–protein crosslinks in intact cells and in vivo. Carcinogenesis 13:1485–1489.

Zhitkovich A, Voitkun V, Kluz T, Costa M. 1998. Utilisation of DNA–protein crosslinks as a biomarker of chromium exposure. Environmental Health Perspectives 106:969–974.