Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

April 2007, 28(2) 287-290 (2007)
For personal use only
Commercial distribution of this copy is illegal

Monitoring of cellular enzymes in the serum of electroplating workers at Coimbatore

C.P. Saraswathy* and M.V. Usharani

Department of Environmental Sciences, Bharathiar University, Coimbatore-614 042, India

(Received: June 03, 2005 ; Revised received: May 15, 2006 ; Accepted: June 17, 2006)

Abstract: *Chromium compounds are potent toxic and carcinogenic substances. With respect to toxicity, hepatic and renal toxicity have been reported both in workers and in animals exposed to chromium (VI). Chromium (VI) compounds induces DNA damage in vivo and in cultured cells as well as the cytotoxicity evaluated by the leakage of lactate dehydrogenase. The present study reports the cytotoxicity of chrome platers who are employed from 8 to 25 years in electroplating industries at Coimbatore, Tamilnadu. Blood samples were collected and estimated for glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and total protein in the serum. The study revealed that there is a significant elevation in the level of LDH, ALP, CPK and transaminases and a decrease in total protein in serum. The results of the study suggests that chromium (VI), a hepatotoxic chemical may perhaps damage the plasma membrane resulting in leakage of enzymes in to the serum of chromeplaters.*

Key words *: Chromium compounds, Serum, GOT, GPT, LDH, ALP, CPK, Protein*

Introduction

Occupational exposure to carcinogenic forms of chromium occurs among workers in several professional groups particularly with high exposure among chromeplaters and stainless steel welders (Zhitkovich *et al.,* 1996). Chromium (III) in its biologically active form) glucose tolerance factor or (GTF) a dinicotinatochromium (III) glutathione - like complex), facilitates interaction of insulin with its receptor site, influencing glucose, protein and lipid metabolism. Thus chromium (III) is essential for animals and human beings (Grevatt, 1998). Evidence from studies on experimental animals shows that hexavalent chromium compounds especially those of low solubility can induce cancer in workers engaged in industries working with chromates, through inhalation and skin contact. Dermal, renal and hepatic toxicity have been reported in workers exposed to chromium (VI) (Leonard and Lawerys, 1980; Verschoor *et al.,* 1988).

Chromium (VI) easily passes through the cell membrane and is subsequently reduced through intermediates to chromium (III) by cellular reductants, the formation of the intermediate oxidation states such as chromium (V) and (IV) may play a vital role in the adverse biological effects of chromium (VI). Further metabolism of chromium compounds in humans has not been well established (Gromadzinska *et al.,* 1996). Welders in India are inclined to possible occupational chromium and nickel exposure. The carcinogenic potential of metals is a major issue in defining human health from exposure (Danadevi *et al.,* 2004).

There are only few reports of the health effects of chronic exposure to hexavalent chromium in developing countries. Coimbatore (Tamilnadu) is an industrial city with a minimum number of 240 chromeplating industries. However no reports of human exposure to hexavalent chromium are available. Hence the present study was under taken to monitor the chromium (VI) induced cytotoxicity in workers exposed to electroplating by evaluating the leakage of few marker enzymes into the serum.

Materials and Methods

Study subjects: Study subjects were one hundred and thirty chromeplaters from a chromeplating unit in the industrial area, Kattoor in Coimbatore, Tamilnadu who were continuously employed in the factory for (8 hrs/day/week) for a minimum of 8 years to a maximum of 25 years. One hundred and thirty controls were the residents of Coimbatore not known to be exposed to chromium or other metals at work or elsewhere and not living in the vicinity of the factory. A short questionnaire was introduced to each worker at the time of the study. The questionnaire was designed to collect demographic characteristics, history of occupation, use of medicines, vitamins and alcohol consumption.

Venous blood samples were collected from control and experimental subjects. Initial steps of sample preparation were performed immediately after collection and all the samples were assigned blind codes before being sent to the laboratory. Becton Dickinson vacationer tubes 15020, Franklin lakes, NJ/07417-1885 were used for obtaining serum. Lactate dehydrogenase activity was assayed by the method of King (1965) where as creatine phosphokinase activity was estimated by the method of Okinaka *et al.* (1961). To evaluate the possible liver function, serum alkaline phosphatase activity was estimated by the method of Moog (1946) as modified by King (1965) using disodium phenyl phosphate as substrate. Serum transaminases GOT and GPT were estimated

Corresponding author: E-Mail: govtarts_saraswathy@yahoo.co.in , Tel.: 0422-24222222, Ext. 395

Table - 1: Selected characteristics of the study population

S. No.	Variable	Controls	Exposed (n = 130)
1.	Age	31 ± 5.77	33.4 ± 5.26
2.	Gender		
	Male	130 (100%)	126 (96%)
	Female	0	4 (4%)
3.	Smoking status		
	Current smokers	58 (45%)	51 (40%)
	Non smokers	72 (55%)	79 (60%)
4.	Alcoholic status		
	Alcoholics	48 (37%)	58 (45%)
	Non alcoholics	82 (63%)	72 (55%)
5.	Years of exposure		
	8-15	-	73 (56%)
	16-25	-	57 (44%)

Table - 2: Activities of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) in control group and chromeplaters

Group	Subjects	AST (IU/l)	ALT (IU/l)
I	Control (n = 130)	19.18 ± 2.14	22.0 ± 1.69
II a	Exposed to chromeplating (8-15 years) (n = 73)	32.92 ± 3.71**	34.34 ± 2.50**
III a	Exposed to chromeplating (16-25 years) (n = 57)	38.62 ± 4.04 **	43.28 ± 1.72**

Values are expressed as Mean ± S.D for 'n' in each group
For statistical evaluation, the values of group I were compared with group II a and III a, ** $p < 0.01$

by the method of King (1965). Total protein in serum were estimated by method of Lowry (1951). The results of the enzyme estimation were tested for significance by Students t-test.

Results and Discussion

Details of the participants namely their age, smoking habit, alcohol consumption and exposure to chromeplating are given in Table 1. Table 2, Table 3 and Table 4 shows the activities of serum LDH, CPK, ALP, GOT, GPT and the level of total protein.

The results of the present study demonstrate that the chromate induced cytotoxicity as estimated by the leakage of LDH, CPK, ALP, GOT and GPT in to the serum. Occupational exposure to chromium (VI) compounds cause dermatitis, penetrating ulcers in the hands and fore arms, perforation of nasal septum and inflammation of larynx and liver.

Heavy metals able to inhibit the activity of membrane bound enzymes and thereby affect the cell function, metabolism and signal transduction. The interaction of metal ions with the lipids of biological membranes might have significant consequences for the structural and functional properties of cells. Cell membranes may be damaged due to peroxidation of unsaturated fatty acids, genetic material may be modified or hormonal composition of a given individual may be changed (Pesti *et al.,* 2000).

The nephrotoxic, hepatotoxic and cardiotoxic actions of hexavalent chromium compounds (Kaufman, 1970; Schubert *et al.,* 1970; Evan and Dail, 1974) as well as their effects on lung, blood and circulation may contribute to the fatal outcome of chromium intoxication (Langard, 1978).

Table - 3: Activities of serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in control group and chromeplaters

Group	Subjects	Lactate dehydrogenase (LDH) IU/l	Creatine phasphokinase (CPK) IU/l
I	Control (n = 130)	250.23 ± 11.15	140.15 ± 6.33
II a	Exposed to chromeplating (8-15 years) (n = 73)	287.66 ± 16.94 **	170.78 ± 8.26 **
III a	Exposed to chromeplating (16-25 years) (n = 57)	371.16 ± 25.2 **	200.39 ± 6.53 **

Values are expressed as Mean ± S.D for 'n' in each group
For statistical evaluation, the values of group I were compared with group II a and III a
** $p < 0.01$

Table - 4: Levels of serum alkaline phosphatase and protein in control group and in chromeplaters

Group	Subjects	Alkaline phosphatase (ALP) IU/l	Protein (g/dl)
I	Control (n = 130)	60.84 ± 5.67	7.80 ± 0.39
II a	Exposed to chromeplating (8-15 years) (n = 73)	70.15 ± 6.24 **	7.52 ± 0.06 **
III a	Exposed to chromeplating (16-25 years) (n = 57)	83.72 ± 7.63 **	6.06 ± 0.11 **

Values are expressed as Mean ± S.D for 'n' in each group
For statistical evaluation, the values of group I were compared with group II a and III a
** $p < 0.01$

With the disruption of the internal cellular structure and with increased permeability or disintegration of the cell wall, many of the cellular enzymes leak in to the interstitial fluid and find their way in to the blood. Elevations in the activities of serum creatine kinase, lactate dehydrogenase, transaminases and alkaline phosphatases when compared to controls could be due to disturbances in heart and liver function. A significant increase in the activity of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in chromium (VI) exposed workers is indicative of disturbance in liver function. Elevated blood transaminases is induced by heavy metals have also been reported (Rajanna *et al.,* 1981). Serum creatine phosphokinase and lactate dehydrogenase are the diagnostic indicators of myocardial functional disorders and quantity of enzyme released from the damaged tissue is a measure of number of necrotic cells. LDH, a cytosolic enzyme is involved in biochemical regulation reactions of the body tissues and fluids. An elevation in LDH in the serum has been observed in ovarian cancer and other malignant conditions (Rohini *et al*., 2004). Creatine kinase activity was reported to be increased 10 fold in lung carcinoma cell lines and in progressive states of cancer (Sell, 1999).

A decrease in the level of protein is observed in chromeplaters exposed to chromium for 8-15 years however not significant, but a significant decrease in the level of protein is observed in chromeplaters exposed to chromium for 16-25 years. The decreased level of serum protein in chromium (VI) exposed group of workers could have been due to proteinuria and nephropathy. Liver is the major site of protein synthesis and hence the observed defect in liver cells would have resulted in decreased synthesis of proteins.

Preliminary studies of cohort workers exposed to nephrotoxic heavy metals have indicated that quantitation of glutathione-s-transferase proteins in the urine constitutes a valuable diagnostic tool (Sundberg *et al*., 1994)

Laborda *et al.* (1986), studied the effect of trivalent and hexavalent chromium compounds on rats transaminase, urea and creatinine levels in the serum of chromium poisoned animals. They have found that enlargement of the proximal tubule of the kidney with a flattening of the epithelial lining and a slight vacuolation of the hepatocytes with the progressive damage in liver, with the enlargement of central veins and sinusoids. Liver morphological alterations were further increased when the period of chromium (VI) exposure was extended at 60 days.

The necrosis in the tissue architecture could have resulted in the leakage of enzymes in to the blood stream. Cytotoxicity caused by potassium dichromate was estimated by the leakage of lactate dehydrogenase from the cells was previously reported by Susa e*t al*., 1996. The cause of the observed enzymological changes are closely connected with the fact that these enzymes are the markers of particular cell organelles. As suggested by Holzer and Duntze (1971), the mechanisms behind the chemical modification of enzymes might include phosphorylation, adenylation, ADP-ribosylation, oxidation of thiol groups and the respective reverse reactions *i.e.* all these reactions remain to be tested for the effect of chromium on them.

In vitro and *in vivo* experiments indicate that unlike inorganic forms of chromium (III), chromium (VI) as a chromate anion can readily enter the red blood cells and once inside the cells, it is reduced and binds to hemoglobin (Lewalter *et al*., 1985; Merrit and Brown, 1995). A significant portion of these chromium-hemoglobin complexes persists for a relatively longer period of time; therefore, a single determination can potentially allow a chromium (VI) exposure assessment for an extended period (Zhitkovich *et al.,* 1996).

To outline the cytotoxicity of chromium (VI) compounds, the molecules of soluble chromium compounds inhaled in to the body and are absorbed by blood through the alveoli. Chromium (III) compounds are generally unable to diffuse through plasma membrane, and chromium (III) has been found to accumulate on the surface where as chromium (VI) is extracellularly reduced to chromium (III) with the participation of a variety of compounds (glutathione, ascorbic acid, serum proteins). This process may initiate a series of free radical reactions and may lead to the formation of a number of active metabolites. Increased peroxidation of biologically active compounds may result in the permanent damage of the structure and the function of the vulnerable organs in the body (Gromadzinska *et al.,* 1996).

Pesti *et al*. (2000), reported that chromium (III) treatment appear to cause a loss of the barrier function of plasma membrane, resulting in the leakage of low molecular mass substances from the cells. He also confirmed that the heavy metal stress induced lipid peroxidation which might contribute to both a fluidifying effect and decreased membrane barrier function of chromium (III) treated cells in agreement with the findings of Howle and Avery (1997) following Ca (II) and Cd (II) treatment.

With respect to chromium (VI) induced cytotoxicity in animals, an inhibition of ALPase in the renal cortex was reported (Kumar and Rana, 1984). On the other hand the elevation of ALPase localized in the cell membrane of liver cells was reported in animals treated with chromate (Chorvatovicova, 1993). These results indicate that chromium (VI) damage the plasma membrane via its ability to increase lipid peroxidation.

Chromium (VI) is extracellularly reduced to chromium (III) with the participation of a variety of compounds, which may initiate a series of free radical reaction and lead to the formation of a number of active metabolites. Because reactive oxygen species produce a number of toxic reactions, studies are currently under way to assay lipid peroxidation and antioxidants apart from estimating the serum marker enzymes.

Acknowledgments

CPS thanks the University Grants Commission, New Delhi and the Principal, LRG Govt. Arts College, Tirupur, for granting FIP study leave.

References

Chorvatovicova, D., E. Ginter, A. Kosinova and Zloch: Effect of vitamin C and E on toxicity and mutagenicity of hexavalent chromium in rat and guinea pig. *Mutat. Res.,* **262**, 41-46 (1991).

Danadevi, K., R. Rozati, B.S. Banu and P. Grover: Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the comet and micronucleus assays. *Mutagenesis,* **19**, 35-41 (2004).

Evan, A.P. and W.E. Dail: The effects of sodium chromate on the proximal tubules of the rat kidney. Fine structural damage and lysocymuria. *Lab Invest,* **30**, 704-715 (1974).

Grevett, P.C.: Toxicology review of hexavalent chromium (CAS No.18540-29-9) U.S.Environmental Protection Agency, Washington, D.C. (1998).

Gromadzinska, J., W. Wasowicz, M. Sklodowska, W. Bulikowski and K. Rydzynski: The Influence of atmospheric chromium on selenium content and glutathione peroxidase activity in blood of tannery workers. *Environ. Hlth. Perspectives,* **104(12)**, 1312-1346 (1996).

Holzer, H. and W. Duntze: Metabolic regulation by chemical modification of enzymes. *Annu. Rev. Biochem.,* **40**, 345-350 (1971).

Howlett , N.G. and S.V. Avery: Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and infuence of plasma membrane fatty acid unsaturation. *Appl. Environ. Microbiol.,* **63**, 2971-2976. (1997).

Kaufman, D.B.: Acute potassium di chromate poisoning in man. *Am. J. Diseases of Children,* **119**, 374 (1970).

King, J.: *In*: Practical Clinical and Enzymology. D. von Nostrund Co. Ltd., London. (1965).

Kumar, A. and S.V.S. Rana: Enzymological effect of hexavalent chromium in the rat kidney. *Int. .J. Tiss. React*., **4**, 135-139 (1984).

Laborada, R., J. Diaz Mayans and A. Nunez: Nephrotoxic and hepato toxic effects of chromium compounds in rats. *Bull. Environ. Contam. Toxicol.,* **36**, 332-336 (1986).

Langard, S.: Chromium. *In*: Metals in the environments. Academic Press. London (1978).

Leonard, A. and R.R. Lawerys: Carcinogenecity and mutagenecity of chromium. *Mutat. Res.,* **76**, 227-239 (1980).

Lewalter, J., R. Korallus and H. Weidemann: Chromium bond detection in isolated erythrocytes: A new principle of biological monitoring of exposure of hexavalent chromium. *Int. Arch. Occup. Environ. Hlth.*, **55**, 305-318 (1985).

Lowry, O.H., Rosebrough, A.L. Farr and R.L. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Merrit, K. and S.A. Brown: Release of hexavalent chromium from corrosion of stainless steel and cobalt-chromium alloys. *J. Biomed. Materials Res.,* **29**, 627-633 (1995).

Moog, F.: Acid and alkaline phospho mono esterase activity of chick embroyos. *J. Cell Comp. Physiol*., **28**, 197 (1946).

Okinaka, S., H. Kumagai, E. Ebashi, M. Sugita, Y. Momoi, Y. Tayokura and Y. Fujie: Serum creatine phosphokinase. *Arch. Neurol.,* **4**, 520 (1961).

Pesti, M., Z. Gazdag and J. Belagyi: *In vivo* interaction of trivalent chromium with yeast plasma membrane as revealed by EPR spectroscopy. FEMS *Microbiology Letters*, **182**, 375-380 (2000).

Rajanna, B., K.D. Chapatwala, D.D. Vaishnav and D. Desaiah: Changes in the ATPase activities in tissues of rats fed on cadmium. *J. Environ. Biol.,* **2**, 1-9 (1981).

Rohini, G., K.E. Sabitha and D. Shyamala: *Bacopa monniera* Linn. extract modulates antioxidant and marker enzyme status in fibrosarcoma bearing rats. *Ind. J. Exp. Biol.*, **42**, 776-780 (2004).

Schubert, S., K. Gebhard and F. Howlein: Renal lesions after serotonin or potassium dichromate injections in sodium loaded and sodium depleted rats. *Virch. Arch. Path. Anat.*, **351**, 66-82 (1970).

Sell, S.: Cancer markers of the 1990's. *Clin. Lab. Med.,* **10**, 27 (1990).

Sundberg, A., K. Vist, E.L. Appel, G. Dallnor and R. Nilsson: Glutothione transferase in urine : sensitive methods for detection of kidney damage induced by Nephrotoxic agents in Humans. *Environ. Hlth. Prospects,* **102(Sup.3)**, 293-296 (1994).

Susa, N., S. Ueno., Y. Furukawa and M. Sugiyama: Protective effect of vitamin E on chromium (VI) induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes. *Arch. Toxicol.,* **71**, 20-24 (1996).

Verschoor, M.A., P. C. Braget, R.F.M. Herber, R.L.Zielhuis and W.C.M. Zwennis: Renal function of chromeplating workers and welders. *Int. Arch. Occup. Environ. Hlth.*, **60**, 67-70 (1988).

Zhitkovich, A., A. Lukanova, T. Popov, E. Taioli, H. Cohen, M. Costa M. and P. Toniolo: DNA – protein crosslinks in peripheral lymphocytes of individuals exposed to hexavalent chromium compounds. *Biomarkers*, **1**, 86-93 (1996).