Keywords: Chromium compounds; Serum; AST; ALT; LDH; ALP; CPK; Total protein

Introduction
Chromium (Cr) is one of the eight metals in the top 50 priority list for toxic substances by the Agency for Toxic Substances and Disease Registry (ATSDR 2003). The majority of Chromium in the environment exists in two valence states: trivalent Chromium Cr (III) and hexavalent Chromium Cr (VI) [1]. Chromium (III) is its biologically active form [Glucose Tolerance Factor or (GTF) a dinicotinato Chromium (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 [2]. In contrast, Cr (VI) compounds can actively penetrate cell membrane through channels for isoelectric and isostructural anions, such as SO42- and HPO42- channels [3,4]. Insoluble chromates are taken up via phagocytosis [5]. Cr (VI) is a strong oxidizing agent, and can be reduced through short-lived Cr intermediates (Cr (V) and Cr (IV)) to the stable trivalent state. The reactions of Cr (VI) with biological reductants, such as ascorbate and thiols, often generate free radicals, which in turn activate O2 and produce reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide [6]. Occupational exposure to Chromium occurs mainly through inhalation and dermal absorption in the working environment, including Chromium compound manufacturing, electropolishing, leather tanning, welding, chrome plating, the manufacture of dyes and pigments, leather and wood preservation, and treatment of cooling tower water. Smaller amounts are used in drilling mud, textiles, and toner for copying machines. Dermal exposure to Chromium may occur during the use of consumer products that contain Chromium, such as wood treated with copper dichromate or leather tanned with chromic sulfate [7]. Prolonged exposure to airborne or solid, liquid Chromium compounds lead to chronic toxic effects on humans. The diseases are nasal septum perforations, ulceration’s of skin surfaces, rhinitis, liver damage, pulmonary congestion, edema, nephritis, intestinal lung and gastric cancers, irritation of gastrointestinal mucosa, chronic total parental nutrition, (Symptoms like weight loss, hypoglycemia), respiratory effects, congestion and hyperemia, chronic rhinitis [8]. There are only few reports of the health effects of chronic exposure to hexavalent Chromium in developing countries. Hence the present study was under taken to monitor the Chromium (VI) induced cytotoxicity in workers exposed to electropolishing by evaluating the leakage of few marker enzymes into the serum. The indicators examined included urinary Chromium (U-Cr), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK). Chromium concentration in erythrocytes was used to monitor hexavalent Chromium exposure CPK and transaminases and a decrease in total protein in serum. The results of the study suggest that Chromium (VI), a hepatotoxic chemical may perhaps damage the plasma membrane resulting in leakage of enzymes in to the serum of chromeplaters.

Materials and Methods

Sample
The study was carried out in August 2010 and involved 84 male workers, who were divided into 2 groups. The first group consisted of 42 workers who were recruited from 12 electropolishing factories in Damietta, Egypt; this group was considered as Chromium – electropolishing workers and subdivided into two groups (II and III) according to the duration of exposure. The second group comprised 42 office workers with no exposure to Chromium and was considered as a reference group. They were working at an office in Damietta city unrelated to the Chromium-electroplating factories. The reference group subjects were matched for age and socioeconomic status (income, area of residence) with the Chromium - electroplating workers. Demographic information and work history and habits (smoking status, alcohol consumption) of all subjects were obtained through a questionnaire.

Laboratory methods
All laboratory equipment was well cleansed by soaking in 10% nitric acid for 24 hours and rinsing thoroughly with deionized water.
[9]. The same cleansing procedure was applied to the polypropylene containers used for venous blood and urine sampling and for storing the serum.

Urine test

A urine sample was collected from the Chromium - electroplating workers in metal-free polyethylene bottles at the end of 8-hour working day for measuring urinary creatinine concentration. All the samples once collected were kept on ice and delivered within the same day to the laboratory with minimal vibration.

Blood test

Samples of 5 mL whole blood were collected in two test-tubes at the end of the working day at the same time as urine sampling. Sample collection followed the guidelines described by Cornelis et al. [10]. In the 1st blood tube serum was separated by centrifugation at 2500 rpm for 20 min at 25°C to assess liver function using standard methods [11,12]. All the biochemical markers were estimated using a random access analyser (RA-50, Bayer).

Measurement of Chromium concentration in erythrocytes and urine

In the 2nd blood tube blood was centrifuged for 10 min at 2000 rpm to isolate erythrocytes for determining Chromium levels. The erythrocytes were washed with Phosphor-Buffered Saline (PBS) for three times. Chromium concentrations in erythrocytes and urine were measured by Inductively Coupled Plasma Method / Mass Spectrometric Method (ICPS - MS). The standard curve was fitted with linear least-squares method. The detection limit of Chromium was 0.2 μg/L. The recovery of standard addition was 95 - 98.8%. The concentration of Chromium in erythrocytes was corrected for hematoctit for each subject. Serum ALT and AST were used to assess hepatic inflammation (kits supplied by Human Gesellschaft fur Biochemica und Diagnostica). Alkaline Phosphatase (ALP) was used to assess choleostasis (kits supplied by Chema Diagnostica). The determination of Lactate dehydrogenase and creatine phosphokinase activity were used to assess Cytotoxicity. The determination of serum total protein was used to assess synthetic function of liver (protein kits produced by Chema Diagnostica). For internal quality control human-based control sera were used (QN.0050CH, Chema Diagnostica).

Statistical analysis

Numerical data were expressed as mean and Standard Deviation (SD). Student t-test was used to compare the mean levels of parameters between the Chromium exposed and reference groups. The chi squared test was used to compare the abnormal frequencies of liver function tests between groups. The level of statistical significance was established at 0.05 with a statistical power of 80%.

Results

Some background parameters of the Chromium- electroplating and reference groups are presented in Table 1. Due to the matching, the mean age of Chromium- electroplating and reference group were similar at 41.6 (SD 2.08) and 43.28 (SD 1.98) years respectively.

Table 2 presents the activities of serum Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) in control group and chromoplaters. There were no significant differences in age, gender, smoking status, alcohol consumption between the two groups (P values > 0.05).

Table 3 presents the activities of serum Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) in control group and chromoplaters. The results of the present study demonstrate that the chromate induced cytotoxicity as estimated by the leakage of LDH, ALP, protein, AST and ALT into the serum.

Table 4 presents the levels of serum Alkaline Phosphatase (ALP) and Total Protein (TP) in control group and chromoplaters. The results of the present study demonstrate that the chromate induced heart and liver function as estimated by the leakage ALP, Total protein into the serum.

Table 5 presents the Chromium concentrations in erythrocytes and urine (in μg/L) in electroplating workers and control group. In this study, we investigated the effects of chronic Chromium exposure on liver in electroplating workers. Chromium concentration in erythrocytes was used to monitor hexavalent Chromium exposure. The results showed that Chromium concentration in erythrocytes in electroplating workers was significantly higher than that in control subjects. The current results indicated that electroplating workers experienced chronic occupational Cr (VI) exposure, which induced oxidative stress and liver damage. 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 [13]. 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 level of serum protein in Chromium (VI) exposed group workers could remain to be tested for the effect of Chromium on them. The decreased level of serum protein in Chromium (VI) exposed group 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. In vitro and in vivo experiments indicate that unlike inorganic forms of Chromium (III), Chromium (VI) as a chromate anion [19]. On the other hand the elevation of ALPase localized in the cell membrane of liver cells was reported in animals treated with chromate [20]. Consistent with previous findings, Chromium concentration in erythrocytes was significantly higher in electroplating workers than that in control subjects in this study. The value (4.19 mg/L) was also comparable to previously reported 4.3 mg/L in electroplating workers [21]. In comparison, higher Chromium concentration (4.9 to 6.1 mg/L) has been reported in chrome-plating workers [22].

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