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Possible Adverse Effect of Chromium in Occupational Exposure of Tannery Workers

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Abstract: Our aim was to investigate the adverse effects of occupational exposure to trivalent chromium. We measured chromium and iron levels in serum and urine and hemoglobin levels in tannery workers and unexposed persons. We studied three groups of subjects. Group 1 included 15 non-smoking male tannery workers highly exposed to chromium from tanning and retanning departments. Group 2 included 14 non-smoking male tannery workers with moderate chromium exposure from dying, drying and finishing departments. Group 3 included 11 healthy, non-smoking male subjects without direct chromium exposure. Higher serum chromium levels were observed in groups 1 and 2 with respect to group 3 (mean values respectively: 0.43; 0.25 and 0.13 $\mu g \cdot l^{-1}$). Urine chromium levels in group 1 were higher than those in controls (mean values: 1.78 and 1.35 $\mu g \cdot l^{-1}$). In group 1 an inverse association was found between serum chromium and urine iron (–0.524), urine chromium and hemoglobin (–0.594) and between the urine chromium to iron ratio and hemoglobin (–0.693, $p<0.05$). The results suggest a chromium adverse effect on iron metabolism, possibly associated with excessive body chromium accumulation. In conclusion, chromium urine test could be recommended for diagnosis of chromium adverse effect on iron metabolism. Further studies are needed to quantify the relationship between urine chromium and hemoglobin metabolism.

Key words: Occupational exposure, Tannery, Chromium, Iron, Urinary excretion

Introduction

Chromium is distributed in its two stable oxidation states +3 and +6. It is found in the city air, at levels of 10–50 $ng \cdot m^{-3}$, and in non-contaminated soil at ranges from 30 up to 300 $\mu g \cdot g^{-1}$. Chromium is also found in food at 5–10 $\mu g \cdot kg^{-1}$. The Mexican Official Norm states 50 $\mu g \cdot l^{-1}$ of total chromium as the maximum permitted level in drinking water, and the normal human serum concentration is about 0.2 $\mu g \cdot l^{-1}$ [1, 2].

Basic chromium (III) sulphate $[Cr(H_2O)_5(OH)SO_4]$ is widely used in the leather industry as the basic tanning agent[3]. The minimum amount of chromium necessary to perform a good tanning is approximately 3 g of Cr_2O_3 for 100 g of leather. Chromium may enter the body by breathing, eating and by direct cutaneous contact, therefore, the tannery workers are exposed to this element, mainly in the inorganic Cr(III) form, or in the protein bound form (leather dust). The recently reported total chromium content in tannery air was 1–54 $\mu g \cdot m^{-3}$ and the evaluated additional element dairy intake for workers was in the range 150–325 μg (for unexposed population < 30 μg)[4]. The International Agency for Research on Cancer has not evaluated chromium (III) compounds as potential carcinogenic agents for humans[5], however positive epidermal tests to high concentration of

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Cr(III) have been observed[1, 6]. Professional exposure to Cr (III) increases the risk of dermatitis, ulcers and perforation of the nasal septum, and respiratory illnesses, as well as increased lung and nasal cancer[7–9]. On the other hand, due to the similarities between Cr(III) and Fe(III) in terms of electrical charge and ion size, the competition of the two ions for transferrin binding sites was observed[10]. The reported evidences for the intracellular Fe (III) deficiency in the presence of Cr (III), are decreased catalase activity as well as excessive excretion of iron and its biological complexes[11].

With the aim to provide further evidence of the possible adverse effects of chromium, in this work we measured chromium and iron levels in serum and urine of tannery workers and in the unexposed persons. The obtained results were compared with hemoglobin levels in these same individuals. The possible relationships between serum and/or urine chromium and other parameters were statistically evaluated.

Subjects and Methods

Group 1

A group of 15 non-smoking male tannery workers from the departments characterized by high exposure to chromium (tanning and retanning are the tannery departments where most chromium exposure exists), in the age range 21 to 43 years (mean 29.2). The selected subjects had a mean working time of 12 years in the above mentioned departments, with a daily exposition of 8 to 10 hours.

Group 2

A group of 14 non-smoking male tannery workers from departments with expected moderate exposure to chromium (dying, drying, finishing are the tannery departments), aged 17 to 38 years old (mean 27.1) and a mean lifetime exposure similar as in group 1.

Group 3 (control)

A group of 11 healthy, non-smoking, male subjects, 21 to 38 years old (mean 29.5), with jobs unrelated to chromium exposure. Their socio-economic and cultural status, as well as their past and current health status was comparable with the other two groups. Diabetes or any other metabolic or degenerative process was not identified in these subjects.

Sample collection

The polypropylene tubes for blood sample collection were previously decontaminated with nitric acid (10%) and then kept in deionized water. In order to avoid possible contamination problems due to the use of stainless needles, 5 ml of deionized water were aspirated to the new syringe and the chromium level in this water was taken as a blank (such procedure was not necessary for iron). The blood volume withdrawn was 5 ml, the first three ml were partly used for the hemoglobin test (partly descarted) and the last portion was collected into decontaminated sample tubes for the determination of chromium and iron in serum.

The 24 h urine samples were collected in the previously decontaminated (see above) plastic containers for determination of chromium and iron. The collection of urine began after discharging the first urine of the day, and continued through the next 24 h including the first urine of the following day. The blood samples were obtained the same morning the 24 h urine was delivered.

Laboratory tests

The determination of chromium and iron in the serum and urine samples was carried out by electrothermal atomic absorption spectrometry (ETA-AAS) with a Model 3110, HGA 600 graphite furnace and AS-60 autosampler (Perkin-Elmer Corp., Norwalk, CT). For chromium analysis, serum and urine samples were diluted three times with deionized water; while iron was quantified in 50 fold diluted serum and two fold diluted urine. A stock standard solutions containing 1000 $mg \cdot l^{-1}$ respectively of chromium and iron were obtained from Sigma Chemical Co. (St. Louis, MO). The instrumental conditions used are presented in Table 1.

Statistics

Descriptive statistics (median, mean, standard deviation), and the correlation coefficients were evaluated, and linear regression analysis was carried out using the Microsoft Excel 97 package. The non-parametric Mann-Whitney U test was used to compare group differences with the use of the Statistica program (Statsoft Inc, Tulsen OK). and a multiple linear regression analysis was performed using Unscrambler 7.5 software package (CAMO Inc., Norway).

Ethics

An informed consent was obtained from each subject. All studies were conducted in conformity with the recommendations from the Declaration of Helsinki. The study was approved by the ethics committee of the Instituto de Investigaciones Médicas, University of Guanajuato.

Table 1. Furnace programmes for chromium and iron determination by ETA-AAS

Step	Temperature (°C)	Ramp (S)	Hold (S)	Ar flow rate (ml/min)
Chromium (i=25mA, λ=357.9nm, δ=0.7nm, WA, 5 μg Mg as chemical modifier)				
1	130	20	30	300
2	150	5	15	300
3	700	5	10	300
4	1400	10	10	300
5	20	1	15	300
6*	2300	0	4	0
7	2600	1	3	300
Iron (I=30mA, λ=248.3nm, δ=0.2nm, PA, 5 μg Mg as chemical modifier, BC)				
1	130	20	30	300
2	150	5	15	300
3	700	5	10	300
4	1300	10	10	300
5	20	1	15	300
6*	2400	0	4	0
7	2600	1	3	300

WA—wall atomisation, PA—platform atomisation, BC—deuterium background correction, *—atomisation step.

Table 2. The results obtained in the samples from the three subjects (each one from different group) using the external calibration (1) and the method of standard addition (2)

	Serum Cr ($\mu g \cdot l^{-1}$)		Urine Cr ($\mu g \cdot l^{-1}$)		Serum Fe ($mg \cdot l^{-1}$)		Urine Fe ($\mu g \cdot l^{-1}$)	
	1	2	1	2	1	2	1	2
1	0.51 ± 0.01	0.52 ± 0.04	1.15 ± 0.03	1.18 ± 0.05	1.45 ± 0.10	1.49 ± 0.26	14.4 ± 0.30	15.1 ± 0.80
2	0.23 ± 0.01	0.26 ± 0.03	1.14 ± 0.02	1.16 ± 0.04	1.59 ± 0.11	1.63 ± 0.25	9.10 ± 0.27	9.02 ± 0.89
3	0.06 ± 0.02	0.10 ± 0.06	1.68 ± 0.03	1.72 ± 0.05	1.64 ± 0.09	1.66 ± 0.30	10.3 ± 0.20	11.4 ± 1.10

The mean values with respective SD obtained for three replicates.

Results

The study was carried out in three groups of male subjects, according to different exposure to chromium, but homogenous in regards to age, life style and socioeconomic and cultural status. Considering the postulated earlier interactions between trivalent chromium and iron or its biological complexes[11, 15] we measured the concentration of these two elements in serum and in 24 hrs urine as well as the blood hemoglobin levels.

The sensitivity for chromium and iron quantification in aqueous solutions by ETA-AAS were 0.05 $\mu g \cdot l^{-1}$ and 1.50 $\mu g \cdot l^{-1}$ respectively. The analysis of each sample was carried out in triplicate using external calibration. As can be observed in Table 2 the relative standard deviation did not exceed 7%, except for the serum samples with chromium levels close to the quantitation limit. A good agreement was achieved between the results obtained by external calibration and by the method of standard addition, confirming the validity of the analytical procedure.

The descriptive statistics of the results obtained in the groups 1 and 2 and the non-exposed group of subjects (group 3) are presented in Table 3. As the distributions of the results from each group showed significant departure from normality, we used the Mann-Whitney U test to compare group differences. We found that serum chromium levels in groups 1 and 2 (mean values respectively 0.39 and 0.25 $\mu g \cdot l^{-1}$) were higher as compared to group 3 (0.13 $\mu g \cdot l^{-1}$) ($p<0.003$ and $p<0.075$). Moreover, we observed higher serum chromium levels in group 1 (high exposure to chromium) than in group 2 (moderate exposure), ($p<0.02$). The differences in urine chromium levels between groups 1 and 2 as referred to group 3 were less marked ($p<0.17$ and $p<0.6$), but showed increased urinary excretion of chromium in group 1 with respect to

Table 3. Descriptive statistics of the results obtained in the two experimental groups and in control individuals

	Cr urine ($\mu g \cdot l^{-1}$)	Cr serum ($\mu g \cdot l^{-1}$)	Fe urine ($\mu g \cdot l^{-1}$)	Fe serum ($mg \cdot l^{-1}$)	Cr/Fe urine	Hb ($g \cdot dl^{-1}$)
Group 1 (n=14)						
Median	1.65	0.40	10.68	1.77	0.14	16.0
Mean	1.71	0.39	11.75	1.87	0.18	16.0
SD	0.72	0.18	3.86	0.37	0.08	0.41
Min. value	0.49	0.12	4.84	1.43	0.05	15.4
Max. Value	2.86	0.72	20.76	2.69	0.29	16.8
Group 2 (n=14)						
Median	1.43	0.24	14.81	2.08	0.11	16.1
Mean	1.43	0.25	13.28	2.13	0.11	16.0
SD	0.45	0.12	3.62	0.32	0.04	0.5
Min. value	0.68	0.05	6.84	1.59	0.05	15.0
Max. Value	2.16	0.55	19.01	2.84	0.20	16.8
Group 3 (n=11)						
Median	1.33	0.10	12.88	1.78	0.08	16.1
Mean	1.35	0.13	13.97	1.82	0.11	16.1
SD	0.52	0.09	5.74	0.36	0.07	0.6
Min. value	0.19	0.03	6.92	1.32	0.02	15.4
Max. Value	2.03	0.32	28.05	2.59	0.27	17.3

group 3 (mean values respectively 1.78 and 1.35 $\mu g \cdot l^{-1}$). In group 1 (high chromium exposure), urine iron (mean 11.33 $\mu g \cdot l^{-1}$) was lower than in group 3 (mean 13.97 $\mu g \cdot l^{-1}$) ($p<0.26$), while the chromium to iron ratio in urine was higher in this group as compared to controls (0.18 vs 0.11 $\mu g \cdot l^{-1}$, $p<0.07$). No statistically significant differences were observed between other pairs of parameters in groups 1 and 2 with respect to group 3.

In further development, the relations between different parameters measured in the three groups of subjects were studied. Statistically significant correlations were found only in group 1, but not in group 2 (moderate exposure to chromium), nor in group 3 (controls). As shown in Fig. 1 a-c and in Table 4, significant correlations were found between serum chromium and urine iron (–0.524), between urine chromium and hemoglobin (–0.594) and between the relation Cr/Fe in urine and hemoglobin (–0.693). After examining Fig. 1, we considered that one of the cases was an outlier, therefore, we repeated the analysis excluding that case. The results for the linear regression model were now: –0.375, –0.530, and –0.669 respectively. Therefore, after the elimination of the outlier, the relations observed were not altered.

The multiple linear regression model analyzing the association of hemoglobin levels with the urine chromium and urine iron: $Hb = b_1 \cdot (Cr_{urine}) + b_2 (Fe_{urine}) + b_3$ showed a statistically significant relation between the urine levels of the two elements and hemoglobin ($b_1 = -0.326 \pm 0.107$, $p<0.01$; $b_2 = 5.14 \cdot 10^{-2} \pm 1.96 \cdot 10^{-2}$, $p<0.02$; $b_3 = 15.98$; squared regression coefficient 0.588, multiple correlation coefficient 0.767).

Discussion

Leon is a major leather-processing center in Mexico with an estimated 10,000 tannery workers. An important risk factor for these persons is occupational exposure to chromium. In a previous study we observed higher serum chromium, and lower insulin, cholesterol and triglyceride levels in tannery workers with respect to the control group and our results[12]. Similar findings are reported in studies on trivalent chromium supplementation[13, 14]. The present work was undertaken in order to provide more information on possible health effects of chromium in tannery workers. The elevated chromium levels in serum observed in the groups 1 and 2 as compared to the group 3 support earlier reports on occupational exposure to this element[12]. We also found higher chromium in serum of workers from tanning and retanning departments (group 1) than in workers from dying, drying and finishing areas (group 2) confirming higher exposure of the group 1 with respect to the group 2 (Table 3). The lower urine iron in group 1 suggests the influence

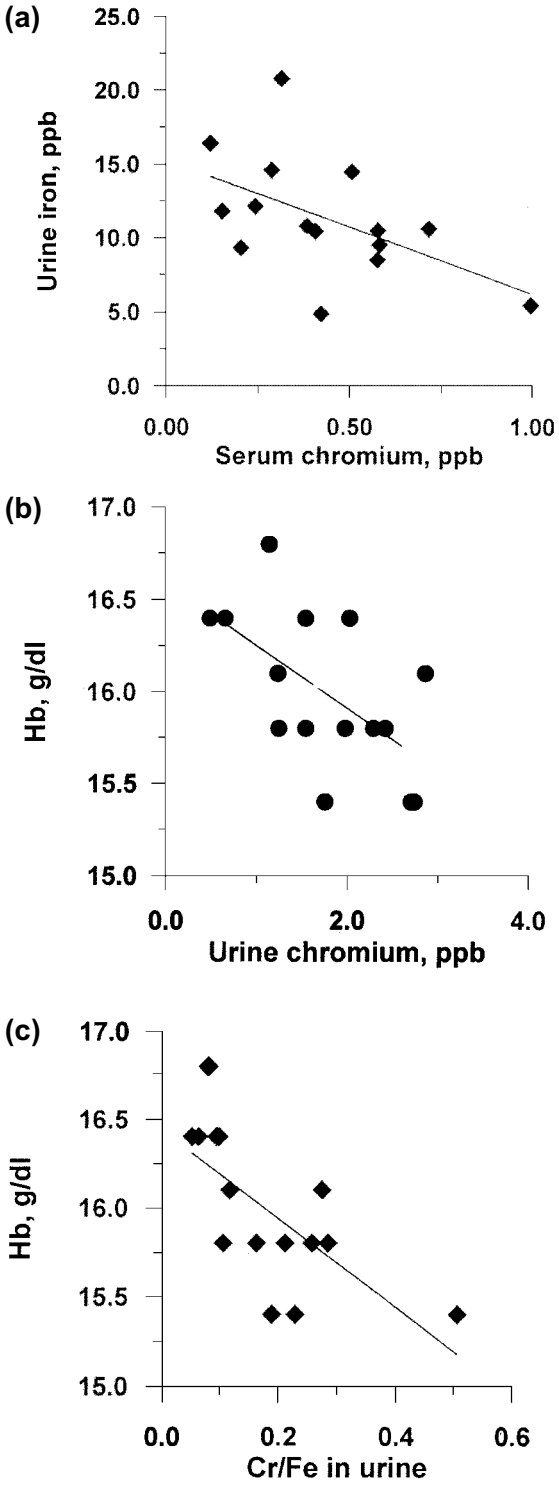

Fig. 1. Relation between the parameters measured in the subjects with high exposure to chromium (group 1).
(solid line - linear regression function, dash line–9% percentile lines)
(a) Chromium in serum vs iron in urine. (b) Chromium in urine vs hemoglobin. (c) Cr/Fe in urine vs hemoglobin.

of chromium on the iron status in these subjects. For better understanding of the possible effect of chromium, we studied the association of serum chromium and the urine chromium/iron ratio with hemoglobin levels. No significant association was found in the groups 2 and 3. Meanwhile in group 1 important inverse relationships were found between serum chromium, and urine iron and hemoglobin (Table 4, Fig. 1). It was previously reported that the concentration of an element in serum reflects the accumulation of this element in the organism[16]. The inverse relationship between serum chromium and urine iron in group 1 (–0.524) suggests that chromium excess in the organism could increases iron requirement. Furthermore, in this group with high chromium exposure, urine chromium correlated inversely with hemoglobin (–0.594), indicating some type of alterations on iron homeostasis. To further evaluate this association, the relation between the chromium to iron ratio in urine with the hemoglobin level was also analyzed and, a higher correlation coefficient was obtained (–0.693, Table 4, Fig. 1c). In other words, in group 1, the hemoglobin level diminished when urinary chromium excretion increased and/or the iron elimination decreased, supporting the postulated effect of high chromium exposure. The results obtained by multiple linear regression confirmed that the hemoglobin level was inversely related with the urinary excretion of chromium, while iron excretion in the exposed individuals decreased with hemoglobin levels. It should be mentioned that the lack of correlation among studied parameters in the groups 2 and 3 indicates that the possible adverse effect of chromium existed only in the subjects with chromium body overload (group 1). Moreover, no statistically significant correlation was observed between chromium serum and hemoglobin in the three groups of study. Although the chromium concentration in serum indicates the element accumulation in the organism, it could not be recommended as a reliable assessment of the health risk related with occupational exposure. Our results seem to indicate that the urine chromium level is a better index of the adverse effects of this element. Moreover, the analysis of blood is an invasive test as compared to urine test and the latter could be easily introduced to the routine health control of the tannery workers. It should be mentioned that the urinary excretion of chromium has already been considered as a possible biological marker in occupational exposure to hexavalent chromium[17–19] but not in tannery workers exposed to trivalent species. We observed the effect of chromium only in the group with high exposure to chromium (tanning and retanning departments), so further studies are needed in order to elucidate the postulated chromium effect on iron

Table 4. The statistically significant relations found between the measured parameters in the three groups of subjects (r- correlation coefficient)

Relation	Group	r, $p<0.05$	Linear regression function
Cr in serum vs Fe in urine	1	–0.375	$Cr_{(s)} = -0.030 \cdot Fe_{(u)} + 0.775$
	2	0.128	$Cr_{(s)} = 0.004 \cdot Fe_{(u)} + 0.195$
	3	–0.005	$Cr_{(s)} = 0.0001 \cdot Fe_{(u)} + 0.129$
Cr in urine vs hemoglobin	1	–0.530	$Cr_{(u)} = -1.03 \cdot Hb + 18.19$
	2	–0.013	$Cr_{(u)} = 0.01 \cdot Hb + 1.22$
	3	–0.085	$Cr_{(u)} = 0.08 \cdot Hb + 0.06$
Cr/Fe in urine vs hemoglobin	1	–0.669	$Cr/Fe_{(u)} = -0.193 \cdot Hb + 3.269$
	2	–0.092	$Cr/Fe_{(u)} = -0.008 \cdot Hb + 0.240$
	3	0.369	$Cr/Fe_{(u)} = 0.044 \cdot Hb - 0.604$

homeostasis and for the estimation of the concentration of urinary chromium, at which the hemoglobin levels start to be altered.

Conclusions

Increased serum chromium was found in tannery workers occupationally exposed to chromium. In group 1 (workers from tanning and retanning departments) an inverse relationship was found between urine chromium and hemoglobin. We also observed lower urinary excretion of iron with increasing chromium serum in these same subjects. The multiple linear regression model constructed for hemoglobin versus urine chromium and iron showed a statistically significant relation between these parameters in the subjects with high exposure to chromium (group 1). These results suggest an adverse effect of chromium which is related with iron metabolism alterations and which can be observed in subjects with excessive chromium accumulation in the organism. We suggest that the chromium urine test could be recommended for diagnosis of the observed adverse effect, but further studies are needed to characterize quantitatively the relation between urinary chromium excretion and the hemoglobin alterations.

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