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Ventilatory Problems and Cytogenetic Changes in Workers Occupationally Exposed to Chromium

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Abstract: Leather tanning is principally the chemical preservation of raw hide by a process takes place in which binding of various chemicals to proteins.Tannery workers have the potential for exposure to many hazardous chemicals, including chromium salts;they are under constant threat of chromium (Cr) toxicity. The present study aimed to study the ventilatory status of workers occupationally exposed to Cr in their working environmental and study the role of cytogenetic changes in the workers with abnormal ventilatory functions (obstructive airways). Forty tannery workers and 59 control subjects were recruited. Physical examinations and Spirometric measurements were performed at the workplaces for all the included subjects. Cytogenetic analysis was carried out with standard procedures on heparinized venous blood leukocytes. Results revealed that Forced Expiratory Volume in one second (FEV1) and Forced Vital Capacity (FVC) were significantly decreased in the exposed workers compared to their controls. Chromosomal aberrations were higher in exposed workers compared to their controls, but not to the level of significance. Sister Chromatid Exchange (SCE) was significantly higher in the exposed workers and in the workers suffering from obstructive airways compared to the controls. This group of workers is likely at high risk of developing respiratory distress and could be up to lung cancer. The workers are in a stage, in which removal from further exposure to Cr is mandatory before carcinogenicity became well established, otherwise, change the environmental conditions to decrease the exposure levels in addition to use the protective measures and devices.

Key words: Chromosomal aberrations · Chromium · Sister Chromatid Exchange · Smoking habits · Ventilatory functions

INTRODUCTION

Leather tanning is principally chemical preservation of raw hide by a process takes place in which binding of various chemicals to proteins. Tannery workers have the potential for exposure tomany hazardous chemicals; such as chromium salts, benzidine based azo dyes, organic solvents (e.g. benzene and formaldehyde), penta-chlorphenol, N-nitroso compounds, arsenic, dimethylformamide and airborne leather dust [1]. These chemicals are potentially irritants and sensitizers for the workers who are frequently exposed to these chemicals for prolonged periods of time [2, 3]. Tannery workers are exposed mainly to chromium salts e.g. potassium dichromate, especially in the leather tanning section. They are under constant threat of chromium (Cr). As Cr is an irritant, it can cause perforation in nasal septum, respiratory problems, dermatitis, gastrointestinal, hepatic andrenal impairments [4]. Direct contact with Cr has the potential to bind with the skin proteins of the workers, producing complex antigen which leads to hypersensitivity [5] and dermatitis [1]. Chromium enters the body in hexavalent form, crosses the cell and is rapidly reduced to chromium trivalent (Cr III) in the body. The health effects of chromium are primarily related to the valence state of the metal at the time of exposure.

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Trivalent (Cr III) and hexavalent (Cr VI) compounds are thought to be the most biologically significant. Cr (III) is an essential dietary mineral in low doses. Cr (VI) compounds are carcinogenic. Cr (VI) is generally considered 1,000 times more toxic than Cr (III) [6]. Chromium hexavalent (Cr VI) is a well-known corrosive, cytotoxic and carcinogenic to humans. Workers exposed to Cr are at high risk of developing nasal and lung cancer [7]. The chromium produces most of its toxic effects by the generation of free oxidizing radicals. Free radicals cause oxidative changes in proteins, various point mutations in DNA, chromosomal damage and adduct formations [8, 9]. Respiratory tract is a target organ for Cr toxicity associated with both acute and chronic inhalations [6]. Obstructive effects of exposure to Cr on lung functions in tannery industry have been reported in few publications. Reduced forced vital capacity (FVC) and an increased prevalence of obstructive lung diseases were found among electro-furnace workers in a ferrochromium plant [10]. Chronic exposure to irritant levels of soluble Cr compounds may cause cough, chest pain, dyspnea and development of asthma. A reduction in the FEV1/FVC ratio may be seen after acute irritation in workers with Cr induced asthma [11]. A high incidence of occupational asthma induced by chromium salts has been detected among workers engaged in tanning, as proved through studying the ventilatory functions of the tannery workers before and after daily work, together with the effect of bronchodilators [12].The possibility of underlying genetic causation cannot be excluded, in addition the extent of exposure at workplace.

The present study aimed to study the ventilatory status of workers occupationally exposed to Cr in their working environmental and study the role of cytogenetic changes in the workers with abnormal ventilatory functions.

MATERIALS AND METHODS

A cross sectional comparative study was conducted on leather tanning workers, comparing to non-exposed workers.

Tanning Processes: Tanning is the chemical process to convert the hides into tanned leather by stabilizing the collagen structure, protecting the leather from enzymatic degradation, enhancing the strength and increasing its resistance to heat, hydrolysis and microbial degradation. Chromium sulphate is the most widely used tanning agent to form cross-linking collagen. They normally used potassium dichromate and phenosulphonic acid formaldehyde, together with mercaptobenzothiazole and meta sodium as a biocide. Sodium bicarbonate is added to stabilize the collagen. Reducing the water content and shaving of the pickled hides are done mechanically [1].

Subjects: In the present study 40 tannery workers and 59 control subjects were included. The workers were from small tannery company in Ain El-Seyra industrial area in Cairo, Egypt. The control subjects were of the same socioeconomic status of the workers, but they never employed in tannery and nor exposed to mutagenic or irritating chemicals in their works. Informed consents were obtained from all the included subjects.

The Workplace Environment: The exposed workers were chosen from the tanning section. All of them were working in ill-ventilated departments. The workers occupationally exposed to mixtures of solvents, dyes, finishing agents and processing chemicals. They also exposed to toxic irritant gases and leather dust. There are several potential sources of air emissions in the leather tanning and finishing industry. Chromium emissions may occur from chromate reduction, handling of basic chromic sulfate powder and from the buffing process. No air emissions of chromium occur during soaking or drying. At plants that purchase chromic sulfate in powder form, dust containing trivalent chromium may be emitted during storage, handling and mixing of the dry chromic sulfate. The buffing operation also releases particulates, which may contain chromium.

Air Sampling: A large number of workers are potentially exposed to chromium. The highest potential exposure occurs in the metallurgy and tanning industries, where workers may be exposed to high air concentrations. Chromium metal is suspended in the air as tiny particles, the concentrations of chromium metal measured in PM_{10} samples. PM_{10} samples provide a better estimate for that than the larger TSP samples. To sample for Cr metal in the workplace, a 37-mm diameter, 5-µm pore size polyvinyl chloride (PVC) filter is used as the sampling medium. Chromium metal concentration was determined after acid extraction of the Cr from the sample [13], using Perkin-Elmer (Norwalk, ct) model 4110ZL atomic absorption spectrometer (AAS), which equipped with auto-sampler, a longitudinal Zeeman back ground correction system and a transversely heated graphite atomizer (THGA). The instrument was interfaced to a personal computer (330-p75, IBM) running the Perkin-Elmer proprietary software package (version 3.1) that controlled the spectrometer.

Ventilatory Function Tests (VFTs): Spirometric measures were performed in the sitting position using a portable spirometer (a calibrated Micro Lab 3000 series by Micro Medical 2td plus printer, Buckingham, UK) according to the criteria of the American Thoracic Society (1995). The ventilatory function parameters in the form of Forced Expiratory Volume in one second (FEV_1), Forced Vital Capacity (FVC) and Peak Expiratory Flow Rate (PEFR) were expressed as the percentage of predicted value for each person after adjustment for age, sex, race and height. The measurement methods were explained in detail to the individuals before testing. Each person was measured three times; the highest values of the FEV1 and FVC were recorded. The percentages under 80% of the predicted value were interpreted as an airway obstruction according to the European Respiratory Society for the year 1993 [14] and according to the Egyptian guidelines for the year 2002[15].

Cytogenetic Study: G-Banding: Cytogenetic analysis was carried out with standard procedures on heparinized venous blood leukocytes. Microculture was set up by the method of Moorhead *et al.* [16]. The incubation period was 72h. G-banding was produced as described by Seabright [17] and Verma and Babu [18]. Metaphase spreads (25-30 cells) were analyzed and any structural or numerical anomalies were recorded according to the ISCN [19].

Sister Chromatid Exchanges (SCEs): To detect SCEs in metaphase chromosomes, 25-30 cells were analyzed. Leukocyte micro culture was set up by the method of Moorhead *et al.* [16] to each culture tube 0.1ml of 5-bromo-2-deoxyuridine solution was added and the culture was incubated in dark for 40–48h, harvesting the slides were done staining the slides according to Verma and Babu [18] in Hoechst 33258 dye for 20 min, then rinsed in distilled water and layered by Mcjlivaine's buffer. The slides were subjected to ultraviolet light for 40min, then washed in distilled water and stained by Giemsa dye. 25-50 metaphases were analyzed under the microscope for each case to detect SCE frequencies.

Statistical Analysis: The collected data was statistically analyzed using SPSS software, version 13.0. The quantitative data were presented in mean ± standard deviation (SD) and the qualitative data were presented in number and percent. Independent t-testwas used to compare between the quantitative data of two groups and for more than three groups Analysis of Variance (ANOVA) and Post hoc least significant differences (LSD) were used. Pearson Chi-square (χ^2) was used for the analysis of qualitative data. Correlation coefficient (r) was used for testing the association between two continuous variables. The significance level was considered at P-value< 0.05.

RESULTS

All the examined subjects were Egyptian males. There was no significant difference between the exposed workers and their controls according to their age (31.6±1.48 and 35.9±2.25 years, respectively) and their smoking habits (16.9% and 17.5% respectively). All the tannery workers were employed for more than 5 years (13.0±1.40 years).The results showed elevation in the concentrations of chromium (Cr) in the suspended dust in all the departments of the factory compared to the outdoor environment. The highest concentration was in Cr tanning section (TWA=5$\mu g/m^3$).

Table 2 shows that FEV1 and FVC were significant decreased in the exposed workers compared to their controls. There was no significant difference between the PEFR of the two examined groups. According to the cytogenetic changes, chromosomal aberrations were higher in exposed workers compared to their controls, but not to the level of significance. The SCE was significantly higher among the exposed workers than in the control group.

Fig. 1 shows a significant increase in the percent of abnormal ventilatory functions among the workers exposed to Cr compared to their controls. In Table 3, ventilatory functions of the exposed workers; whether smokers or non-smokers; were significantly decreased than that of their controls.

Table 4 shows that SCE was significantly higher in the workers with normal ventilatory functions compared to the normal controls and higher in the workers with obstructive airways compared to normal and abnormal controls. Chromosomal aberrations showed no significant difference between the workers and the controls (Table 2), as well as between the workers and the controls according to their ventilatory functions.

In the exposed workers after controlling for smoking index, the VFTs and chromosomal aberrations were not significantly correlated with the duration of exposure. While, there was significant correlation between the SCEs and the duration of exposure (r = 0.4, P< 0.01). Fig. 2 is an example of chromosomal aberrations and Fig. 3 shows multiple SCEs.

Table 1: Chromium concentrations in different departments of the tannery plant compared to the outdoor environment.

Sites of measurement	Cr in air (µg / m³)	References
Outdoor Environment	6.5µg / m³	Present study
Preparatory section	9.0µg / m³	
Cr tanning section	59.0 µg / m³	
Vegetable tanning section	15.0µg / m³	
Finishing section	29.0µg /m³	
Occupational Safety and Health Administration (Air of workplace)	5 µg/m³ as CrO3/m³	Regulation; PEL† for chromic acid and chromates, (8-hour TWA)
	500 µg/m³ as Cr	PEL for Cr(II) and Cr(III) compounds (8-hour TWA)
	1,000 µg/m³ as Cr	PEL for chromium metal and insoluble compounds (8-hour TWA)
National Institute for Occupational Safety and Health (Air of workplace)	1 µg/m³ as Cr	Advisory; TWA (10-hour) for chromic acid and all Cr(VI) compounds
	500 µg/m³ as Cr	Advisory; TWA (10-hour) for chromium metal and Cr(II) and Cr(III) compounds

†PEL (permissible exposure limit): highest level of chromium in air, to which a worker may be exposed, averaged over an 8-hour workday.

TWA (time-weighted average): TWA concentration for a normal workday and a 40-hour workweek to which nearly all workers may be repeatedly exposed.

Table 2: Comparison of the ventilatory functions and cytogenetic changes in the two examined groups

	Control group (59)		Exposed group (40)		Independent t-test	
Item	Mean	SD	Mean	SD	T-test	P-value
FEV1%	99.1	11.7	77.4	19.0	6.398	P<0.0001
FVC%	85.3	12.2	72.5	15.2	4.609	P<0.0001
PEFR%	82.1	18.2	77.7	16.1	1.203	NS
Chromosomal aberrations	3.1	0.5	4.2	1.7	0.584	NS
SCE	7.2	0.4	8.6	0.4	2.404	P<0.05

Table 3: Ventilatory functions of the two examined groups according to their smoking habits

	Control group (59)		Exposed group (40)		ANOVA	
	Non-smokers (49)	Smokers (10)	Non-smokers (33)	Smokers (7)		
Item	X±SD	X±SD	X±SD	X±SD	F-ratio	P-value
FEV1% LSD	101.5±7.79 (Esm, Enon)	96.5±14.64 (Esm, Enon)	77.1±18.64 (Csm, Cnon)	77.5±19.56 (Csm, Cnon)	16.888	P< 0.0001
FVC% LSD	89.0±11.1 (Csm, Esm, Enon)	81.3±12.29 (Cnon, Esm, Enon)	72.3±14.82 (Csm, Cnon)	72.6±15.83 (Csm, Cnon)	8.936	P< 0.0001
PEFR%	83.4+2.2	76.4+2.8	78.6+4.5	77.2+3.2	0.966	NS

N.B. Esm= exposed smokers, Enon= exposed non-smokers, Csm= controls smokers, Cnon= controls non-smokers

Table 4: Comparison of chromosomal aberrations and SCEs among the four subgroups

	Controls		Workers		ANOVA	
Item	Normal (a)	Obstructive airways (b)	Normal (c)	Obstructive airways (d)	F-ratio	P-value
SCE LSD	7.1 ± 0.4 (c, d)	8.0 ± 0.3 (d)	8.8 ± 0.6 (a)	9.7 ± 1.1 (a, b)	7.27	P < 0.01

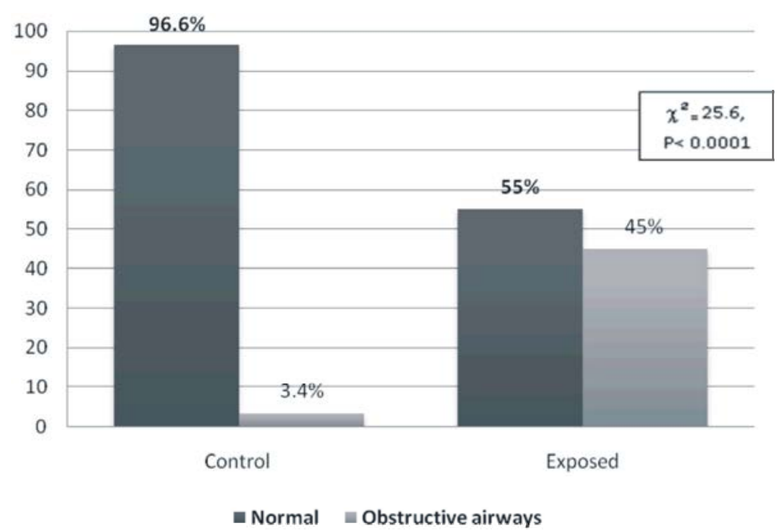

Fig. 1: Distribution of COPD among the examined groups

Fig. 2: Chromosomal aberrations

Fig. 3: SCE

DISCUSSION

The high morbidity among the tannery workers are due to the long-term exposure to air pollutants such as leather dusts, ozone, chromium, lead, nitrogen (NO_2), oxides of sulphur (SO_2), hydrogen sulphide (H_2S), particulate matter less than 10µm (PM_{10}) and volatile organic chemicals (VOC),either individually or in combination, evolved from the leather tannery effluents [20]. In fact various studies have demonstrated that inhalation of these air pollutants, may be linked to the increased prevalence of allergic diseases and can enhance the airway response to inhaled allergens in atopic subjects inducing asthma exacerbations. So, tannery workers were exposed to chemical splashes, dust and mist, leather dust, paint spray and organic vapors [1]. The Occupational Safety and Health Administration (OSHA) have established an 8-hour time-weighted average (TWA) exposure limit of 5µg/m^3 air of Cr (VI). This is a considerable reduction from the previous permissible exposure limit (PEL) of 52µg/m^3. The National Institute for Occupational Safety and Health (NIOSH) has recommended a 10h TWA exposure limit for all Cr (VI) compounds of 1µg/m^3. The recommended exposure limit for Cr metal, Cr (II) and Cr (III) compounds is 500µg/m^3 as 10h TWA. On the basis of current evidence, NIOSH considers all Cr (VI) compounds potential occupational carcinogens [21].

The environmental total Cr in the tannery workplace in the present study was ranged between 6.5 to 29.0µg/m^3 air. These results confirm the statement by Kolomaznik *et al*. [2] that tannery workers have a high risk of exposure to metal salts (mainly chromates) at their workplace. Tannery workers are also continuously exposed to Cr III, which appear to be associated with both acute and chronic health problems [1,4, 8]. Serum levels of Cr metal had not been measured in the present study, which is a limitation in our study, but air Cr in the tannery workplace were measured as index of their occupational exposure. Many studies, found high blood Cr concentration in occupationally exposed tannery workers, to approximately two fold the control [22, 8], other studies showed an increase many fold in plasma and urine Cr in the exposed tannery workers in comparison with controls [23, 24]. Also steel workers occupationally exposed to Cr had significant higher levels of urinary Cr in comparison with their controls [9].

The chemicals used in tanneries alter the structure of animal hide and therefore may have a damaging effects on the function and the structure of the worker's skin [1] and similar other tissues. Cr is the major industrial contact allergen and is widely used in leather tanning and dyeing works. There have been very few publications on the respiratory problems in leather tanning workers exposed to Cr. Short to moderate duration exposure to low levels of Cr VI compounds generally causes mild irritation, accumulation of macrophages, hyperplasia, inflammation [5] and decreased lung function [25]. Chronic bronchitis, emphysema, pulmonary fibrosis and impaired lung function have been observed in nickel-chromium welders and foundry workers [26, 27]. The ventilator functions; FVC and FEV1 are considered good predictors for health of the respiratory system [28]. The present study revealed a significant decrease in the ventilatory function tests among the tannery workers in comparison with the control group. Most of the mean levels of the exposed group were below the cutoff points set for normal values for pulmonary function parameters. The percentage of abnormal ventilatory functions in the studied groups showed that, the highest percentage of workers who suffer from airway obstruction was 45% compared to the controls (3.6%). This difference is statistically significant. These findings come in accordance with previous studies in Egypt that linked occupational exposure to multiple irritant pollutants of the respiratory tracts in tannery industry and the development of chronic obstructive bronchitis in 11.2%, 9.1% and 13.7% of the tanning [12, 29, 30], respectively. Fidan *et al*. [27] detected that abnormal ventilatory functions were decreased in 35% of the welding workers exposed to welding fumes including chrome and other gases.

Data on respiratory function in leather tanning workers could be comparable to those of leather shoe workers and welders as tanning workers are exposed to multiple irritants chemicals, organic dusts and Cr. It was proved that exposure to organic dust significantly reduces the ventilatory function in the exposed workers [31]. Abdallah *et al.* [32] concluded that shoe workers are at risk of respiratory affection due to their exposure to dust of tanned leather. It was proved also that, lung functions of the workers in welding exposed to fumes rich in Cr were significantly lowered in them after welding compared to controls and the ventilatory defects in the welder group were obstructive rather than restrictive [27, 33]. In addition, Billings and Howard [34] concluded that the association of welding fumes with obstructive airway diseases could be as important as that of smoking. Cigarette smoking is recognized as an important factor in the development of chronic air way obstruction [35], but in the present study, smoking didn't express an important synergistic effect in the exposure to the environmental pollutants in the examined tannery workers. The ventilatory functions, of the exposed workers; whether smokers or non-smokers were significantly decreased than that of their controls. In addition, the exposed non-smoker group of tanning workers showed more deterioration in their pulmonary functions compared to the smoker controls.

In addition to the ventilatory problems detected inthe present study, we also observed a genotoxic effect of the workplace pollutants, mainly chromates among the tannery workers. Chromosomal aberrations and sister chromatid exchange (SCE) were used in the current work for expression of the DNA damage in tannery workers. The cytogenetic study, including the chromosomal aberrations and the SCEs, is considered as one of the few direct methods that can measure mutation or other forms of DNA damage in humans exposed to potential mutagens or carcinogens, by measuring the gross changes occurring in the DNA, which can be visualized by looking at the chromosomes through a light microscope. SCEs have been defined as the interchange of DNA replication products at apparently homologous chromosomal loci.Also, it was reported that SCE has served as a test for the mutagenic and carcinogenic potential of many chemical and physical agents both *in vivo* and *in vitro* [36]. The current results revealed that, SCEs was significantly increased in the tannery exposed groups (8.8±0.6) when compared with the control values (7.2±0.4) and SCEs scored its highest values in the exposed group with obstructive airways (9.7±1.1), this value is highly significant, when compared with the control groups. This was consistent with Hassanein *et al.* [9], they proved that SCE in steel workers exposed to Cr were significantly higher compared to their controls and it was significantly correlated with the urinary Cr levels of the exposed workers. The same meaning was proved in the present study, the SCE was significantly correlated with the duration of exposure of tannery workers, as increasing in the duration of exposure could result in increasing the exposure period to Cr. Also, Ambreen *et al.* [8] and Shellappa *et al.* [37] found another biomarkers for DNA damage, which was increased 45 with increasing duration of exposure in tannery workers. This could be explained by the facts that tannery workers were exposed to high levels of Cr in form of Cr (III) and Cr (VI). It was revealed that Cr (VI) can cross cell membranes but it has a short intracellular life, reducing within minutes to hours" to the potentially carcinogenic trivalent state. The reduction of Cr (VI) in the cell is associated with the generation of reactive oxygen species (ROS) band radicals and also lower valence forms which form stable complexes with intracellular macromolecules which can account for the DNA damage [8, 9, 24].

Moreover the inhaled particulate forms of Cr (VI), was found to be associated with mild irritation, accumulation of macrophages, hyperplasia, inflammation and decreased lung functions [25]. Generally, diameter of these metal particles is smaller than 80 nm. Studies not only had shown that particles, the diameter less than 10μm, could pass the larynx and penetrate trachea, but also revealed particles whose diameter less than 2.5μm can be deposited deep in alveolar ducts and alveoli [38]. Inhalation of metal particles had the capacity to lose electrons and generate ROS. ROS could cause damage to membrane lipid of pulmonary capillary endothelial cells, alveolar epithelial cells and basilar membrane cells. It also leads to lung epithelial lining fluids reduction, increase damage of surfactant protein and finally induces alveolar collapse, atelectasis and alveolar ventilation dysfunction [39, 40]. In addition, the inhaled particulate forms of Cr (VI), was found to be associated with increased lung cancer risk [9, 41, 42]. This is obvious in the present study, where SCEs scored its highest significant values in the exposed group with abnormal VFTs. So this group of tannery workers was likely at risk of developing respiratory distress up to lung cancer.

In conclusion, tannery workers had significant expo sure of Cr at their workplace and 45% workers had decreased ventilatory functions. The level of DNA damage; measuredby chromosomal aberrations and SCE. SCE was significantly higher in the exposed workers and in the workers suffering from obstructive airways compared to the controls. This group of workers is likely at high risk of developing respiratory distress and could be up to lung cancer. Thus, the tannery workers is under high risk and in a stage in which removal from further exposure to Cr is mandatory before carcinogenicity become well established. The workers should be made aware of the health hazards due to Cr. Provision of personnel protective equipment, regular occupational biomonitoring of air levels of Cr and gases, at the workplace and the nearby surroundings that can be used as a tool to reduce the exposure risk to Cr and other pollutants in the tannery workers, particularly those with SCE. However, further studies are also needed in order to elucidatethe hazardous effects of trivalent Cr in a broader population of tanning workers.

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