

## Evaluation of Genotoxicity in Tannery Industrial Workers Exposed to Chromium using Comet Assay

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Received: 1.10.2016 | Revised: 18.10.2016 | Accepted: 21.10.2016

### ABSTRACT

*A study was conducted on a group of employees from tannery industries in Telangana state were tested for comet tail lengths that are well established as indicators of early biological effects. To investigate whether occupational exposure to chromium is genotoxic, a total of 92 workers and 80 control groups of individuals in the age group of 16 to 65 years and 6-30 yrs of chromium exposure were recruited; a questionnaire based survey was conducted. In the present study, the assessment of Primary DNA damage hosted by peripheral blood leukocytes of workers employed in tannery based industry was performed using the alkaline comet assay, the tail length and long-tailed nuclei thereby being the primary outcome of the measure. A significant increase in the incidence of DNA damage was observed in the experimental subjects when compared to their respective controls. The results obtained in this investigation indicate that tannery workers seem to be facing the occupational hazard of genotoxicity due to inhalation and handling of chromium.*

**Key words:** DNA, Genotoxicity, Assay, Chromium

### INTRODUCTION

Leather tanning is the process of converting raw hides of skins into leather. Tanning is essentially the reaction of collagen fibers in the hide with tannins, chromium, alum or other chemical agents. Approximately 90% of all leather is produced by chrome tanning. Basic trivalent chromium compounds are used in the leather production as a chelating agent to stabilize collagen fibers in the animal skin, providing it with the known thermal and hydro resistance of leather. Chrome tanning is still the most economically advantageous method to produce good quality leather. The

heaviest metal exposure occurs in the workplace among occupationally exposed groups. A person spends, on average, one-third of his life at his workplace and therefore the environment in which he works can be a major factor in determining health.

Leather production includes many operations with different exposures, which can be harmful for the health of the workers, and particularly be carcinogenic. Some compounds in the tanning process are considered as probably being carcinogenic to humans (some benzene-based dyes and formaldehyde).

**Cite this article:** Rudrama, K.D. Evaluation of Genotoxicity in Tannery Industrial Workers Exposed to Chromium using Comet Assay, *Int. J. Pure App. Biosci.* 4(5): 168-174 (2016). doi: <http://dx.doi.org/10.18782/2320-7051.2383>

An important health risk factor for the tannery workers is occupational exposure to chromium, mainly in the organic Cr (III) form or in the protein bound-form (leather dust). There are several potential sources of air emissions in the leather tanning and finishing industry such as chromium emissions that may occur from chromate reduction, handling of basic chromic sulfate powder and from the buffing process. Cr and Cr compounds have been tested for genotoxicity in a variety of short-term tests using different end-points<sup>5,8,19</sup>. Moreover, there are reports on positive genotoxic effects in populations exposed to Cr<sup>4,28,18,27</sup>. Chromium causes a variety of DNA lesions such as DNA strand breaks, SCEs and mutations<sup>19,5,22</sup>. The oxidation state is the most important parameter for chromium toxicity. Cases of nasal cancer were also reported among these workers, exposed to a variety of forms of chromium, including Cr (VI) and Cr (III) compounds. There is increased incidence of health problems in tannery workers<sup>26</sup> lung cancer<sup>22</sup> and dermal problems<sup>20</sup>.

Chromium may enter the body by inhalation, ingestion and by direct cutaneous contact. Professional exposure to Cr (III) increases the risk many diseases. In the present study, the assessment of Primary DNA damage hosted by peripheral blood leukocytes of workers employed in tannery industry was performed using the alkaline comet assay, the tail length and long-tailed nuclei thereby being the primary outcome of the measure. Comet assay can sensitively detect DNA single strand break and alkali-labile site<sup>31</sup>. It was used in this study to examine lymphocyte DNA damage of CT users. This technique has suggested a positive role of the comet assay in the human monitoring of DNA damage from environmental and /or occupational exposure to carcinogenic and mutagenic agents, and has been shown to be a very sensitive method to detect genetic damage at the individual cell level and in human biomointoring .

## MATERIALS AND METHODS

### Analysis of chromium content:

A total of ninety two (92) tannery workers were selected and correspondingly 82 control subjects were included in the present investigation. From the total number of subjects blood was collected for estimation of chromium. An ultra mass 700 inductively coupled plasma mass spectrometer (Varian, Australia) was utilised<sup>14</sup>. The blood was homogenized for 10 min by mechanical shaking. Aliquots (0.5 ml) were diluted 1:9 with a solution of 4.5 ml of deionized water containing ammonia (0.07 M), Triton X-100 (500 mg/l) and Na<sub>2</sub>-EDTA (500 mg/l). The sample digests were filtered with Wattman paper several times. The diluted digests were measured directly by IC-PMS and concentrations of Cr were quantified as micrograms per liter (mg/l).

**Chemicals:** Agarose-normal melting, agarose-low melting, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, disodium ethylenediaminetetraacetic acid (disodium EDTA), tris, sodium hydroxide , sodium dodecyl sulphate / sodium lauryl sarcosinate, tritron X 100, trichloro acetic acid, zinc sulphate, glycerol, sodium carbonate, silver nitrate, ammonium nitrate, silicotungstic acid, formaldehyde. Subject Recruitment and Sample Collection: The study was conducted on 92 workers aged 16- 66 years from Telangana. The control groups consisted 80 people, aged 16-66 with no history of exposure to clastogenic and/or aneugenic agents and socio-economic level also similar to that of experimental subjects. At the time of sample collection (3ml/individual) all the tannery workers signed a term of informed consent and replied to Questionnaires elaborated to determine the profile and habits of study population. The protocol has been approved by local ethical committee. The exposed workers to chromium, the duration of service was taken

more than five years. Peripheral blood samples ( $V = 5$  ml) were collected under sterile conditions by venipuncture into heparinized tubes for comet assay<sup>30</sup>.

#### Single Cell Gel Electrophoresis (SCGE):

The comet assay was conducted under alkali conditions according to Singh *et al.*<sup>30</sup>. All chemicals were obtained by Sigma. Two microlitre of whole blood were suspended in 0.5% low melting agarose and sandwiched between a layer, of 0.6% normal melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. The slides were kept on ice during the polymerization of each gel-layer. After the solidification of 0.6% agarose layer, the slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris-HCl, 1% Triton X-100 and DMSO 10%) at 4 °C. After 1hr, the slides were placed in the

electrophoresis buffer (0.3 M NaOH, 1 mM Na<sub>2</sub>EDTA, pH 10) for 20 min at room temperature to allow for DNA to unwind. The buffers were then chilled and the electrophoresis was performed at 300 mA and 19V in a horizontal electrophoresis platform for 20 min. The slides were neutralized with Tris-HCl buffer (pH 7.5) and stained with 10% ethidium-bromide for 10 min. Each slide was analyzed by using Leitz Orthoplan epifluorescence microscope. For each subject 50 cells were analyzed by automatic digital analysis system Comet assay II (Perceptive Instruments Ltd., Suffolk, Halstead, UK), determining tail length and tail moment (tail length×tail % DNA/100). DNA damage was further quantified by visual classification of cells into categories of ‘comets’ corresponding to the amount of DNA in the tail according to Anderson *et al.*<sup>2</sup>.

## RESULTS

**Table 1: Characteristics of the exposed workers and control subjects**

Group	No. of sample	Age (Years) Mean + SD	Duration of service
Control	80		-
Smokers		31.6 ± 4.1	
Non Smokers		41.2 ± 6.0	
Exposed	92		
Smokers		36.0 ± 5.01	9.4 ± 6.1
Non Smokers		42.0 ± 7.14	11.0 ± 8.1

**Table 2: Frequencies of comet tail length in tannery workers and controls**

Treatment	Controls	Exposed group				
		1-7yrs	8-14yrs	15-21yrs	22-28yrs	1-28yrs
No. of samples	80	30	20	22	20	92
<b>Controls</b>						
Smokers	0.52±0.04	-	-	-	-	-
Non smokers	0.68±0.08	-	-	-	-	-
<b>Exposed</b>						
Smokers	0.92±0.512	3.68±0.82	3.96±0.96	4.92±1.20*	5.98±1.46*	4.63±1.36*
Non smokers	0.82±0.402	3.02±0.58	3.21±0.72	3.34±1.09*	4.24±1.73*	4.03±1.27*

\*P<0.05

**Chromium concentration in whole blood**

The heavy metals Cr were estimated in the whole blood of 92 tannery workers and 82 controls by ICP-MS. Welders showed significantly higher Cr concentrations when compared with controls as analysed by ANOVA (Cr, 132.85 versus 15.62 mg/l;  $P < 0.001$ ).

**Comet assay:**

The frequency of comet tail length in tannery workers is increased to 4.63 % from 0.92 % in control. The difference in the frequency of comet tail length between control and treated groups were statistically found to be significant ( $P < 0.01$ ). The effect of occupational exposure in tannery workers on the level of DNA damage in lymphocytes of study group was assessed by the comet assay. The exposed workers showed significantly higher levels of DNA damage than controls. The range of mean tail moment (MTM) was higher than controls respective. There was significant difference MTM ( $P < 0.01$ ) between experimental and controls. The slides were screened with help of fluorescent microscope available at Indian Institute of Chemical Biology, Tarkana, Hyderabad. ANOVA and simple linear regression analysis were performed to assess the association between endpoints and independent variables.

**DISCUSSION**

The toxicity of chromium (Cr) in occupational settings has been essentially focused on the hexavalent form of the metal, a Group 1 known human carcinogen according to IARC (International Agency for Research on Cancer) classification. Nevertheless, hexavalent chromium has no toxic action until it is reduced inside the cell to lower oxidation states, the most stable being the trivalent form, Cr(III). Therefore, the Cr(III) may be the ultimate intracellular toxicant. The hexavalent form is regarded as the primary toxic threat, due to its easy passage through biological membranes in contrast with the trivalent form, considered quite less toxic due to less efficient membrane passage. Nevertheless, trivalent chromium absorption has been demonstrated in workers exposed to this valence state, which indicates that the rate of

uptake of Cr (III) by the cells may be slower, but effective in chronic occupational exposure settings. Organic complexes of trivalent chromium are absorbed to a greater extent than inorganic compounds, due to a better solubility in biological membranes. For some occupations involving trivalent chromium exposure, increased risks for some cancers have been suggested, but the epidemiological data do not permit discrimination between effects due to hexavalent chromium or other carcinogenic agents in simultaneous exposures.

The aim of our study was to investigate the genotoxic effects associated with occupational exposure to Cr and Ni by analysing DNA damage in blood leukocytes of welders in India, using the alkaline single cell gel electrophoresis (SCGE) assay, also known as the Comet assay. The SCGE assay has been found to be a very sensitive method for measuring DNA damage<sup>30,31</sup>. It is a quick, reliable and fairly inexpensive way of measuring DNA damage. It has a further advantage that the observations are made at the single cell level.

The results of the present study showed significant increase in smokers in the frequency of comet tail length than in non smokers earlier we reported similar observations were reported in workers occupationally exposed to lead<sup>24,25,26</sup>.

It is not possible to measure the concentration of hexavalent chromium in biological material because its oxidizing properties mean that it readily reacts with a number of substances present in the human body. In this situation the observation that only hexavalent chromium is able to pass cell membranes is of great value. In this manner chromate ions also enter erythrocytes. They are reduced there and bound to constituents of the cell. In contrast, trivalent chromium ions do not succeed in passing cell membranes property of chromate ions is especially valuable for the biological monitoring of exposed workers. Indeed, our results indicate that the Cr concentration in blood seems to be a suitable parameter of chromate exposure.

The absorption of Cr and Ni quantified in whole blood samples of welders in the present study was found to be significantly higher than in the control population. Similarly, the average blood and plasma Ni concentration was elevated among electrolysis workers). In this study, welders showed 8- to 9-fold higher amounts of Cr in whole blood of exposed subjects were reported in comparison with controls<sup>9</sup>. A recent study indicated that chronic occupational exposure to trivalent Cr can lead to a detectable increase in lymphocyte DNA damage which correlates with significant exposure of cells to the metal<sup>16</sup>. The Comet assay is increasingly being used to monitor genotoxic effects in occupationally exposed humans<sup>11</sup>. In the present study, a significant increase in comet tail lengths was observed in tannery when compared with controls by the Comet assay. These results indicate that the level of exposure in the workplace is sufficiently high and also highlights the sensitivity of the assay used.

These results confirm the statement by Kolomaznik et al.<sup>13</sup> 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<sup>6,12,1</sup>. 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 than control<sup>1,23</sup>, other studies showed an increase many fold in plasma and urine Cr in the exposed tannery workers in comparison with controls<sup>16,8</sup>. Also steel workers occupationally exposed to Cr had significant higher levels of urinary Cr in comparison with their controls<sup>29</sup>.

Also, Ambreen *et al.*<sup>1</sup> and Shellappa *et al.*<sup>29</sup> 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<sup>1,23,16</sup>.

This result seems to indicate a higher genotoxic risk in the tannery population. However, leather processing involves a considerable number of potentially genotoxic substances which may be contributing to the cytogenetic lesion reported<sup>29,10,19,3</sup>. The increases frequencies of comet tail length which reflect the cumulative effect of the complex mixture of chemical agents involved in leather tanning industry procedures (which had been reported previously

### CONCLUSION

Our findings conclude that chromium exposure causes instability of the genetic material in the workers and can be taken as an indication that these individuals have increased cancer risks. To enable a better assessment of the relative importance of dermal versus inhalation exposure, further quantitative data on uptake of chromium dust via the skin would be needed. Quantitative data on dermal uptake of chromium among exposed workers, relative to the inhalatory dose will nable a health risk assessment. This would require well-designed field studies with small groups of exposed workers either (i) solely skin exposed or (ii) solely with inhalation and (iii) a group with both dermal and inhalatory exposure.

### Acknowledgement

The author (KRD) thankful to University Grant Commission for the award of UGC BSR Faculty fellowship, to Prof. Sugita Mathur, Head, Department of Zoology for providing necessary laboratory facilities

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