2011 International Conference on Bioscience, Biochemistry and Bioinformatics
IPCBEE vol.5 (2011) © (2011) IACSIT Press, Singapore

Enhanced Micronuclei in Exfoliated Buccal Cells of Tannery Workers Exposed to Chromium III (Cr III) in South India

Sabari Guru Vasudevan, Sudha Sellappa, Shibily Prathyumnan, Shyn Joseph, Kripa S Keyan
Department of Biotechnology
Karpagam University
Coimbatore, Tamilnadu, India

Abstract —**Trivalent chromium used in tanning industry is an environmental contaminant that acts as a carcinogen towards humans and animals. The carcinogenic potential of metals is a major issue in defining human health risk from exposure. In the present investigation, 84 tannery workers and 52 control subjects with similar mean ages, smoking prevalences and alcohol consumption were enrolled for DNA damage analysis in buccal cells by Micronucleus assay (MN). Workers showed a significant increase in micronucleated cells compared to controls with respect to their smoking habits and alcohol consumption, age and years of exposure. The current study suggests that chronic occupational exposure to Chromium during tanning could lead to increased levels of DNA damage.**

Keywords-trivalent chromium; genotoxicity; micronucleus assay; tannery workers.

I. INTRODUCTION

Chromium is a widely used industrial metal distributed in its two stable oxidation states +3 and +6 [15]. Elemental chromium (0) does not occur naturally on earth. Commercial applications of chromium compounds include tanning (III), corrosion inhibition, plating, glassware-cleaning solutions, wood preservatives (VI), manufacture of safety matches, metal finishing (VI), and the production of pigments (III, VI) [4].

Chromium has been recognized as one of the most effective tanning agents and has been widely employed in the leather industry since its discovery more than 100 years ago. Since then, some 85 % of the leather produced worldwide is tanned with chromium salts, either alone or in combination with other tanning agents. Chrome tanning is economically advantageous and provides good quality leather, and is not likely to be replaced by the existing alternative tanning agents in the near future.

Cr and Cr compounds have been tested for genotoxicity in a variety of short-term tests using different end-points [24, 25, 14]. Moreover, there are reports on positive genotoxic effects in populations exposed to Cr [8, 26, 21, 3, 9]. Workers occupationally exposed to Cr are considered to be at an elevated risk for developing cancer [23, 11].

Basic chromium (III) sulfate $[Cr(H2O)_5(OH)SO_4]$ is widely used in the leather industry as a chelating agent to stabilize collagen fibers in the animal skin, providing it with the known thermal- and hydro-resistance of leather [6]. 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). Sensitivity to trivalent compounds is much less frequent, but some workers may react to high concentrations of these compounds. Occupational exposure represents the main source of human contamination by chromium.

The present study aimed to investigate the genotoxic effects associated with occupational exposure to Cr (III) on South Indian tannery workers using the Micronucleus test (MN test). This test allows the detection of both clastogenic and aneugenic agents [22]. The influence of confounding factors like age, smoking, alcohol drinking, duration of exposure on the differences in DNA damage was also analyzed.

Although it is well established that tobacco smoking causes lung cancer, cancer at other sites and several other adverse health effects; less information is available concerning the outcome of combined exposure to cigarette smoke (CS), and other agents [1]. The present study analyzed the synergistic genotoxic effect of smoking and alcohol consumption among tannery workers.

II. MATERIALS AND METHODS

A. Study Subjects

Buccal cell samples were obtained from full-time tannery workers (n =84) directly involved in the chromium tanning process. We also collected buccal cells and urine from control individuals (n=52) not known to be exposed to either environmental or occupational carcinogens. The experimental and control groups was further branched as smokers (64 and 28), non-smokers (20 and 24), alcoholics (57 and 27) and non-alcoholics (27 and 25) respectively. The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers and those who consumed >120gm of alcohol/day were considered as alcoholics in both groups. Individual questionnaires were filled by all subjects, the questionnaire covered standard demographic questions (age, genetic disorders, number of X-ray diagnoses, vaccinations, medication, smoking, alcohol, etc.) and occupational questions (years of exposure). We

ensured that the workers and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The workers and control subjects were informed of the objectives of the work, and gave expressed informed and written consent to participate in this study before the collection of urine or buccal cell sample. The samples of urine and buccal cells were coded and the anonymity of the workers and control population was guaranteed. The institutional ethical committee approved the research procedures used in this study.

B. Cell Sampling and Preparation

Buccal cells (BCs) were collected from consented volunteers at the end of the work shift according to the criteria established by Tolbert et al [20]. Prior to BC collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Buccal cell samples were obtained by rubbing the inside of both cheeks using a cyto brush. The cells were collected in tubes containing 3ml sterile saline.

C. Micronucleus Assay

The MN test was carried out on buccal epithelial cells of 84 workers and 52 controls, selected randomly from the total number of subjects, according to the method of Rajeswari et al [19]. Oral buccal cells obtained were smeared on a pre-cleaned slide, fixed in methanol and stained with 2% Giemsa. A total of 2000 cells per individual were scored for analysis of micronuclei.

D. Statistical Analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for biomarker. The significance of the differences between control and worker end-point means were analysed using Student's t-test, whereas simple and multiple linear regression analyses were performed to assess the association between end-points and the independent variables. All calculations were performed using Windows statistical package, version 11.5 (IL, USA). Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking and age) was determined using analysis of variance (ANOVA).

III. RESULTS

The distribution of subjects with respect to age, smoking, alcohol consumption and years of exposure is given in Table I. The two groups studied had similar demographic characteristics.

A. Micronucleus Frequency in Buccal Cells

The frequency of micronuclei (MN) was studied in 84 workers and in 52 controls. Workers revealed a significant induction of MN when compared with controls (7.19 vs. 4.03;) (Table I). Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei (11.85 and 12.05) vs. (5.75 and 6.59) when compared to non smokers and non alcoholics (6.45 and 6.29) vs. (2.87 and 2.88). Workers who are smokers and alcoholics showed a highly significant increase ($p<0.05$) in MN frequency when compared to all other groups and subgroups (Table 1).An increase in MN frequency with an increase in duration of exposure was observed in workers (11.02 versus 9.12) (Table II). An age-dependent increase in MN frequency was also noted both in controls and exposed (2.61 vs. 2.04 and 6.85 vs. 5.12) (Table II).

IV. DISCUSSION

The extensive use of chromium in industrial settings has elicited concern over the safety of workers and surrounding population. The trivalent form of chromium which is extensively used in leather tanning may directly react with the genetic material and has also been shown to generate oxidative damage *in vitro*. Although The International Agency for Research on Cancer has not evaluated chromium (III) compounds as potential carcinogenic agents for humans [12], the risk involved in chronic exposure is uncertain.

Present study reports an elevated MN frequency among Cr (III) exposed south Indian tannery workers. The current analysis suggests that tannery workers under their particular conditions of exposure (tobacco smoke and alcohol) reveal clear evidence of genotoxicity in buccal epithelial cells when evaluated by MN test. Previous investigations reporting genotoxic effects in workers of tanning industry using the MN test are scanty. Our study revealed a significant induction of MN in workers when compared to controls with respect to their age and years of exposure.

A clear increasing trend in MN incidence was evident in a large study designed specifically to assess the effect of ageing on chromosome damage [2]. Bukvic et al [18] also demonstrated a strong correlation between age and MN frequency and suggested that chromosome loss is a determining factor in this increase. Although the link between smoking and cancer is strong and exposure to genotoxic carcinogens present in tobacco smoke has been convincingly demonstrated [13, 10], and the same convincing association is apparent when assessing biomonitoring studies of genotoxicity. Fenech [17] showed that, after adjustment for age and sex, individuals with high cigarette usage [16] had statistically greater MN compared to non-smokers. An increase in MN has been observed in alcoholics consuming alcoholic beverages but not in abstainers of a year or more [5, 7]

Therefore our results demonstrate that MN assay performed in exfoliated buccal mucosa cells is an ideal methodology to measure potential risk related to Cr (III) exposure. However, the results of this study are not enough to establish any causal connection, although there is experimental evidence that supports the genotoxicity of Cr (III). Also, the possibility of unrecognized confounding factors is inevitable in studies such as this.

Workers in many occupational settings are exposed to certain genotoxic agents. These workers may not be aware that they have been exposed to genotoxic agents nor do they

know the type and amount of agent to which they have been exposed. Therefore, there is a need to educate those who work with heavy metals about the potential hazard of occupational exposure and the importance of using protective measures.

TABLE I. FREQUENCY OF MICRONUCLEI WITH RESPECT TO SMOKING HABIT AND ALCOHOL CONSUMPTION IN CONTROL AND TANNERY WORKERS

Characteristics			Sample size (n=136)	Age (years)	Exposure duration (years)	No. Of Cigarettes/ day	Alcohol (gms/day)	MN (M±SD)
Controls	Total		52	37.63 ± 8.44	-	-	-	4.03 ± 1.65
	Smoking	Yes	28	38.03 ± 7.53	-	9.17 ± 2.49	-	5.75 ± 1.40*
		No	24	36.45 ± 8.28	-	-	-	2.87 ± 0.78
	Alcohol consumption	Yes	27	34.55 ± 8.10	-	-	195.55 ± 44.77	6.59 ± 1.15*
		No	25	36.52 ± 8.22	-	-	-	2.88 ± 0.83
Exposed	Total		84	37.48 ± 8.60	18.03 ± 8.31	-	-	7.19 ± 1.15*
	Smoking	Yes	64	38.23 ± 8.19	18.81 ± 6.31	9.21 ± 2.29	-	11.85 ± 2.06*
		No	20	36.5 ± 9.32	14.65 ± 8.38	-	-	6.45 ± 1.19
	Alcohol consumption	Yes	57	39.71 ± 8.08	19.17 ± 8.49	-	196.31 ± 40.78	12.05 ± 2.10*
		No	27	35.89 ± 9.44	15.70 ± 8.51	-	-	6.29 ± 1.13

*p<0.05

TABLE II. FREQUENCY OF MICRONUCLEUS WITH RESPECT TO DURATION OF EXPOSURE AND AGE IN CONTROLS AND TANNERY WORKERS

Characteristics		Control (n=52)		Exposed (n=84)	
		Sample size	*MN frequency (M±SD)*	*Sample size*	*MN frequency (M±SD)*
Age (years)	<35	21	2.04 ± 0.04	38	5.12 ± 1.10
	≥35	31	2.61 ± 0.15*	46	6.85 ± 1.23*
Exposure (years)	<15	-	-	32	9.12 ± 2.02
	≥15	-	-	52	11.02 ± 1.57*

*p<0.05

ACKNOWLEDGMENT

The authors are grateful to the authorities of Karpagam University and Dr. K Sasikala, Unit of Human Genetics, Department of Zoology, Bharathiar University for their open handed support and encouragement. We also thank the volunteers of the study for their cooperation.

REFERENCES

[1] A. Hedenstedt, D. Jenssen, and B. M. Lidestein, "Mutagenicity of fume particles from stainless steel welding", Scand J Work Environ Health, vol. 3, pp. 203-211, 1977.

[2] C. Bolognesi, C. Lando, A. Forni, E. Landini, R. Scarpato, L. Migliore, and S. Bonassi, "Chromosomal damage and ageing: effect on micronuclei frequency in peripheral blood lymphocytes", Age Ageing, vol. 28, pp. 393-397, 1999.

[3] D. Benova, V. Hadjidekova, R. Hristova, T. Nikolova, M. Boulanova, I. Georgieva, M. Grigorova, T. Popov, T. Panev, and R. Georgieva, (2002) "Cytogenetic effects of hexavalent chromium in Bulgarian chromium platers", Mutat. Res., vol. 514, pp. 29–38, 2002.

[4] D. G. Barceloux. "Chromium", J. Toxicol. Clin. Toxicol., vol. 37, pp. 173-194, 1999.

[5] E. Castelli, P. Hrelia, F. Maffei, C. Fimognari, F. G. Foschi, F. Caputo, G. Cantelli-Forti, G. F. Stefanini, and G. Gasbarrini, "Indicators of genetic damage in alcoholics: reversibility after alcohol abstinence", Hepatogastroenterology, vol. 46, pp. 1664-1668, 1999.

[6] E. L. Tavani, and C. Volzone, (1997) "Adsorption of Cr(III) from a tanning wastewater on kaolinite", J. Soc. Leather Technologists and Chemists, vol. 81, pp. 143–148, 1997.

[7] F. Maffei, G. C. Forti, E. Castelli, G. F. Stefanini, S. Mattioli, P. Hrelia, "Biomarkers to assess the genetic damage induced by alcohol abuse in human lymphocytes", Mutat. Res., vol. 514, pp. 49-58, 2002.

[8] F. Sarto, R. Tomanin, L. Giacomelli, G. Iannini, and A. R. Cupiraggi. "The micronucleus assay in human exfoliated cells of the nose and mouth: application to occupational exposures to chromic acid and ethylene oxide", Mutat. Res., vol. 244, pp. 345–351, 1990.

[9] G. Quievryn, E. Peterson, J. Messer and A. Zhitkovich, "Genotoxicity and mutagenicity of chromium(VI)/ascorbate generated DNA adducts in human and bacterial cells", Biochemistry, vol. 42, pp. 1062–1070, 2003.

[10] H. F. Stich, W. Stich, and B. B. Parida. "Elevated frequency of micronucleated cells in the buccal mucosa of individuals at high risk for oral cancer: betel quid chewers", Cancer Lett., vol. 17, pp. 125-134, 1982.

[11] H. J. Gibb, P. S. Lees, P. F. Pinsky, and B. C. Rooney, "Lung cancer among workers in chromium chemical production", Am. J. Ind. Med., vol. 38, pp. 606-610, 2000.

[12] International Agency for Research on Cancer, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Chromium, Nickel and Welding", IARC, Lyon, vol. 49, pp. 213-214, 1990.

[13] International Agency on Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon: IARC; 2002.

[14] K. Kusal Das. "A comprehensive review on nickel (II) and chromium VI toxicities - possible antioxidant (Allium sativum Linn) defences", Am. J. Med. Sci., vol. 2, pp. 43 -50, 2009.

[15] M. D. Cohen, B. Kargacin, C. B. Klein, and M. Costa. "Mechanisms of chromium carcinogenicity and toxicity", Crit. Rev. Toxicol., vol. 23, pp. 255–281, 1993.

[16] M. Fenech, "Cytokinesis-block micronucleus cytome assay", Nat. Protoc., vol. 2, pp. 1084-1104, 2007.

[17] M. Fenech, "The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations", Mutat. Res., vol. 285, pp. 35-44, 1993.

[18] N. Bukvic, M. Gentile, F. Susca, M. Fanelli, G. Serio, L. Buonadonna, A. Capurso, and G. Guanti, "Sex chromosome loss, micronuclei, sister chromatid exchange and aging: a study including 16 centenarians", Mutat. Res., vol. 498, pp. 159-167, 2001.

[19] N. Rajeswari, Y. R. Ahuja, U. Malini, S. Chandrashekar, N. Balakrishna, K. V. Rao, and Ashok Khar, "Risk assessment in the first degree female relatives of breast cancer patients using the alkaline comet assay", Carcinogenesis, vol. 21, pp. 557–561, 2000.

[20] P. E. Tolbert, C. M. Shy, J. W. Allen, "Micronuclei and other nuclear anomalies in buccal smears: methods development", Mutat. Res. vol. 271 pp. 69–77, 1992.

[21] R. R. Tice, "The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells", in Enivironmental Mutagenesis, vol III, D. H. Phillips and S. Venitt, Eds. Bios Scientific Publishers, Oxford, UK, 1995, pp. 315–339.

[22] S. A. Salama, M. Serrana, and W. W. Au, "Biomonitoring using accessible human cells for exposure and health risk assessment", Mutat. Res., vol. 436, pp. 99–112, 1999.

[23] S. De Flora, "Threshold mechanisms and site specificity in chromium (VI) carcinogenesis", Carcinogenesis, vol. 21, pp 533–541, 2000.

[24] S. De Flora, M. Bagnasco, D. Serra, and P. Zanacchi. "Genotoxicity of chromium compounds: a review", Mutat. Res., vol. 238, pp. 99-172, 1990.

[25] T. J. O' Brien, S. Ceryak, and S. R. Patierno. "Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms", Mutat. Res., vol. 533, pp. 3-36, 2003.

[26] Y. J. Choi, Y. M. Kim, and C. W. Cha. "A study on sister chromatid exchanges in lymphocytes in some metal plating workers", Korea Univ. Med. J., vol. 24, pp. 249–257, 1987.