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INCIDENCE OF MICRONUCLEUS IN EXPOLIATED EPITHELIAL CELLS OF TANNERY WORKERS EXPOSED TO CHROMIUM

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ABSTRACT

Chromium (Cr) is widely used industrial chemical. The carcinogenic potential of metals is a major issue of defining human health risk from exposure. A study was conducted on a group of population exposed to chromium in tannery industries in Telangana State. The exposure poses a risk to human health and development of several type of cancers. A total of 92 male workers in tannery industry and equal number of control subjects situated at IDA, Jedimelta, Ranga Reddy, District of Telangana. A questionnaire based survey was conducted and buccal smears were collected form oral cavity and analysed for nuclear abnormalities. A high frequency of karolyses was observed among chromium exposed workers. The high percentage of nuclear anonealies were noted in exposed group when compared with longer duration. Smokers in chromium exposed group enhanced the frequency of micronuclei in buccal cells. The present results clearly revealed that chronic occupational exposure to Cr during leather processing could lead to increased levels of DNA damage in exfoliated buccal cells.

KEYWORDS :

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.

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-tanned leather tends to be softer and more pliable than vegetable tanned leather. Chrome tanning is cost-effectively beneficial and provides superior leather, and is not likely to be replaced by the existing alternative tanning agents in the near future. 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 [O' Brien et. al., 2003, Choi et. al., 1987, Fenech et. al., 2007]. Moreover, there are reports on positive genotoxic effects in populations exposed to Cr [Sarto et.al., 1990; Mikoczy et.al., 1994; Salama et.al., 1999; Benova et.al., 2002; Quievryn et.al., 2003]. Workers occupationally exposed to Cr are considered to be at an elevated risk for developing cancer [De Flora 1990, Gibb et.al., 2000]. Basic chromium (III) sulfate [Cr(H2O)5(OH)SO4] iswidely used in the leather industry as a chelating agent [Tavani, and Volzone 1997]. The minimum amount of chromium necessary to perform a good tanning is approximately 3 g of Cr2O3 for 100 g of leather. The nature of the salt of chromium present is also an important factor of absorption and toxicity. 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).

Chromium causes a variety of DNA lesions such as DNA strand breaks, SCEs and mutations [O' Brien et.al, 2003; Wu et.al 2002; Tolbert et. al. 1992; Choi et.al., 1987]. 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 (Rudrama devi and Dilip reddy, 2015) lung cancer (Rastogi et al., 2007) and dermal problems (Priti Sharma et al., 2012).

In the present investigation studies were carried in exfoliated buccal cells objected from tannery industrial workers. Degenerative nuclear changes, such as micronuclei (MN), binucleates (BN) karyorrhexis (KR) and Karyolsis were analysed in chromium exposed population.

Materials and methods

The study was carried out in 92 chromium exposed workers. The control group consists of 80 healthy individuals with no exposure to any toxicant or any other chemicals participants are informed about the study, asked to sign the consent form and complete the questionnaires to obtain necessary information ion their life style and personal habits (age, working duration smoking habits, health etc.)

Preparation of buccal cell sampling :

Prior to buccal cell collection the tannery workers were advised to rinse their mouth thoroughly with water to remove unwanted debris. Sterile wooden spatula was used to obtain cell samples from buccal mucosa. The mucosa was transfer to Eppendff of tubes with PBS at Ph. 7.0 and centrifused for 10 min at 1500 rpm. Supernatant was removed and replaced with fresh PBS solution. This process was repeated thrice and the pellet was smeared on clean slides. Smears were air dried and fixed in 1:3 acetic acid and methanol fixative for 10 min. slides are air dried and stained with 2% Giemsa for 10 Min. the slides are observed under microscope.

Scorning criteria for buccal cytome assay from each sample three slides were scored Nuclear abnormalities were classified according to the Tolbert et. al. (1992). These criteria are intended to classify buccal cells into categories that distinguish between "Normal and Abnormal" based on their aberrant nuclear morphology. The abnormal morphologies are due to the DNA damage and cell death.

Scoring method and statistical analysis:
To determine the frequency of vaiorus cell types, about 1000 cells were scored for the presence of micronuclei cell, binucleared cells, karryoorhectic and karyoloytic ells. All the data were expressed as the Mean Standard Deviation. The synergistic effect between smoking and exposure were tested with a two way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student + test.

Results
Table 1 and 2 shows the main characteristics in controls cases studies. The mean age group of the selected workers belongs to the range from 31.6 ± 4.1 to 41.2 ± 6.0 in control group and from 36.0 ± 5.01 and 42.0 ± 7.14 in the exposed group they belonged to the similar social economic status. The characteristics of the studied group are mentioned in Table 1. The cytological observations reveals micronuclei and binucleated cells of buccal smears. The mean value of micronuclei in smokers was 8.20 ± 1.06 as against 6.12 ± 1.03 in non smoker exposed group. The mean value of binucleated cells in subjects without smoking was 8.62 ± 6.12 as against 14.12 ± 1.04 in subjects with a habit of smoking higher cells of Karyorrhix cells (KRC). The values were significant higher in smoker of exposed subjects compared to non smokers exposed group. This indicating this habit of smoking enhanced the man values of KRC and KLC nuclear anomalies when compared to control values. The frequency of micornucleate cells, binucleate cells, Karyohexis and Karyolysis cells were compared in duration of exposure less than 5 years and in ten years exposure and it more signficiationly higher in ten years of service workers.

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: Cytological observations in control and exposed group

Individuals	MNC	BNC	KRC	KLC
Control Smokers (N=36) Non Smokers (N=44)	3.18 ±0.06 2.06 ±0.02	4.80 ±0.42 2.80 ±0.08	10.20 ± 0.18 8.20 ±0.80	31.20 ±0.12 20.12 ±1.02
Exposed Smokers (N=44) Non Smokers (N=48)	8.20 ± 1.06* 6.12 ± 1.03	7.62 ±1.01* 5.62 ±1.05	14.12 ± 1.04* 8.62 ±0.62	44.32 ±0.06* 30.12 ±0.10
Duration of exposure 5 years (N = 44) 10 years (N = 48)	5.02 ±0.62 9.80 ±0.80	5.60 ± 0.40 11.20 ± 0.80	16.0 ±1.10 26.0 ±1.08	3.0 ± 1.08 46.0 ±0.96

*P<0.05

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. A study on chromate production workers found that trivalent chromium showed no association with an increased risk of lung cancer, when adjusted for hexavalent chromium exposure and smoking [Gibb et.al., 2000]. Studies of other occupational exposure to hexavalent and trivalent chromium found increased risk of lung cancer, but the studies did not discriminate between hexavalent and trivalent exposure.

There are no reliable data for the settling of tolerable daily intake for oral exposure; therefore, no sample risk characterization for oral exposure can be done. Cytogenetic biomarkers are the most frequently used end point in human biomonitoring studies and are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability. 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) 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. Fenech [Cohen et.al., 1993] showed that, after adjustment for age and sex, individuals with high cigarette usage [Fenech 1993] had statistically greater MN compared to non-smokers.

The harmful effect of dust in various forms of human health have been already demonstrated (Gutherie 1992, Dong et al. 2006). The MN Test scientifically approved is important in demonstrating the genotoxic effects of harmful substance on health (Nerseyan, 2005 Fenech et al. 2007) such as measuring genotoxicity in petrol station employees (Celik, 2003) agricultural workers (Pastor et. al, 2002) Cigarette smokers and tobacco users (Kul et. al. 1997, Prioita et. al. 2006) workers exposed to pesticides (Pastor et al. 2002) timber dust (Celik and Kanik 2006) Ozone and Cancer patients (Chen et. al. 2006 Bloching et al. 2000).

The micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factor that may influence the rate of DNA damage such as incidence of micronuclei in humans (Celik et. al. 2003) reported that cigarette smoking significantly increase the frequencies of nuclear abnormalities in both controls and exposed subjects. Increase in exposure to toxic chemicals such as formaldehyde and benzeze induces a significant increase in the bucall cell micronuclei (Titenko Holland et. al. 1996, Suralles et al. 1997) copper smelters (Lewinska et. al 2007), shoe factory workers (Gian et. al. 2009).

A significant proportion of induced micronuclei may be the result of aneugenic effects of metals. Micronuclei results either from lesions/adducts at the level of DNA or chromosomes, or at the level of proteins directly or indirectly involved in chromosome

segregation. 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). 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. Since trivalent chromium may carry a risk to human health, it should be used more carefully and further investigation on the DNA damage is recommended.

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 enable 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.

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References:

1. Benova D., 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.
2. Bloching M, Hofmann A, Lautenschlager C, Berghaus A and Grummt T 2000 Exfoliative Cytolog buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN Oncol 36 : 550-555.
3. Celik A, Cava T and Ergene Gozukara S 2003 Cytogenetic Biomonitoring in petrol station attention Micronucleus test in exfoliated buccal cells. Mutagenesis 18: 417-421.
4. Chen C, Arjomandi A, Qin H, Balmes J, Tager I and Holland N 2006 Cytogenetic damage in buck and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis 21:131.
5. Choi Y. J., Y. M. Kim, and C. W. Cha. 1987 "A study on sister chromatid exchanges in lymphocytes in some metal plating workers", Korea Univ. Med. J., vol. 24, pp. 249–257.
6. Cohen M.D., B. Kargacin, C.B. Klein, and M. Costa, 1993 "Mechanisms of chromium carcinogenicity and toxicity", Crit. Rev. Toxicol., vol. 23, pp. 255–281.
7. De Flora S., M. Bagnasco, D. Serra, and P. Zanacchi, 1990 "Genotoxicity of chromium compounds, a review", Mutation Research, vol. 238, pp. 99–172.
8. Dong F, Deng J, Pu ZX and John H 2006 Pulmonary alveolar macrophage cytotoxicity investigation shape mineral dusts. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 23 848 51.
9. Fenech M, "Cytokinesis-block micronucleus cytome assay", Nat. Protoc., vol. 2, pp. 1084-1104, 2007.
10. Fenech M. Bolognesi C, Krisch Volders M, Bonassi S, Zeiger E, Knasmuller S and Holland N 2001 Harmonistation of the micronucleus assay in human buccal cells A Human Micronucles (HUMN) inititative commencing in 2007. Mutagenesis 22:3-4.
11. Fenech M., 1993 "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.
12. Guthrie Jr GD 1992 Biological effects of inhaled minerals. Am Mineral 77: 225 243.
13. Gibb H. J., 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.
14. Levinenska AJ, Savian A, Talaksa G, Boeniger MF, Suruda A and Schulte PA 1997 the Uility of epit Micronuclei in the assessment of intermittent exposure. Biomarkers 2:135-138.
15. Mikoczy Z., A. Schütz, and L. Hagmar, 1994 "Cancer incidence and mortality among Swedish leather tanners", Occup. Environ. Med., vol. 51, pp. 530-5.
16. Nersesyan AK 2005 Nuclear buds in exfoliated human cells. Genet Toxicol Environ Mutagen 88.
17. O' Brien T. J., S. Ceryak, and S. R. Patierno. 2003 "Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms", Mutat. Res., vol. 533, pp. 3-36.
18. Pastor S, Creus A, Xamena N, Siffel C and Marcos R 2002. Occupational exposure to pesticides damage : Results of a Hungarian population study using the micronucleus assay in lymphocytes Envrion Mol Mutagen 40 : 101 -109.
19. Prioia NK, Paszkiewicz GM, Nasca MSS, Franke GE and Paul JL 2006 Smoking and smokeless to associate human buccal cell mutations and their association with oral cancer A review. Cancer Biomarkers Prevent 15: 1061 1077.
20. Priti Sharma, vipin Bihari, Sudhir k Agarwal, and sudhir K. Goyal, 2012. Genetic predisposition on dermal problems in hexavalent chromium exposed population journal of nucleic acids. http://dx.doi.org/10/1155/2012/968641.
21. Quievryn G., E. Peterson, J. Messer and A. Zhitkovich, 2003 "Genotoxicity and mutagenicity of chromium(VI)/ascorbate generated DNA adducts in human and bacterial cells", Biochemistry, vol. 42, pp. 1062–1070.
22. Rastogi SK, Keshavachandran C, Mahdi F Pandey A. 2007. Occupational Cancers In Leather tanning industries : A short review Indian journal of occupational environ. Med 11(1):3-5.
23. Rudrama devi K and Dilip reddy K, 2015. Effect of occupational exposure on the incidence of health profile in tannery workers. Int. J. Envi. Sci 6(2):295:299.
24. Salama S. A., M. Serrana, and W. W. Au, 1999 "Biomonitoring using accessible human cells for exposure and health risk assessment", Mutat. Res., vol. 436, pp. 99–112.
25. Sarto F., R. Tomanin, L. Giacomelli, G. Iannini, and A. R. Cupiraggi. 1990 "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.
26. Surralle's J. Carbonell E, Marcos R, Derassi F, Antoccia A and Tanzarella C, 1992. A case study on the improvement of micronucleus test in cultured human lymphocytes Mutagenesis, 7 : 407-4010.
27. Tavani E. L., 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.
28. Titenko – Holland N, Moore LE, Smith MT Measurement and characterization of micronuclei in exfoliated human cells by fluorescence in Situ hybridization with a centrometric probe. Mut Res. 313:39-50.(1994).
29. Tolbert P. E., C. M. Shy, J. W. Allen, 1992 "Micronuclei and other nuclear anomalies in buccal smears: methods development", Mutat. Res. vol. 271 pp. 69–77.
30. Tolbert PE, Shy CM and Allen JW 1992 Micronuclei and other nuclear anomalies in buccal smea development. Mutat Res. 271-:69-77.
31. Wu F.Y., F.J. Tsai, H.W. Kuo, C.H. Tsai, W.Y. Wu, R.Y. Wang, and J.S. Lai, 2000 "Cytogenetic study of workers exposed to chromium compounds", Mutation Research, vol. 464, pp. 289–296.