

Ethnobotanical Leaflets 12: 524-31. 2008.

Biological Monitoring and Chromosomal Aberration of Workers in Rubber Industry

Indra, M. and Bhuvaneshwari, V.

Department of Biochemistry, Avinashilingam University for Women

Coimbatore- 641 046

Bhuvana.adu@gmail.com

Issued 25 July 2008

Abstract

The genotoxic and biochemical characteristic effect of environmental pollutants generated in a rubber tyre industry workers was investigated on biochemical characteristic of 15 workers exposed for different periods and compared with an equal number of unexposed controls matched in respect of age, sex, social status, period of exposures, smoking habits and drug intake, if any. Chromosomal aberrations (CA), hematological parameters, selected bio-chemical parameters and enzymic antioxidants were analysed. All the parameters showed a significant increase ($p < 0.05$) in the exposed sample compared with controls. The environmental pollutants generated in rubber industry were thus found to be genotoxic and tobacco smoke was found to enhance the genotoxic effect.

Key Words: Biomonitoring, Chromosomal aberration, Rubber industry.

Introduction

Exposure in the rubber industry are multitudinous and have given rise to several occupational health concerns, among others, cancer, cardiovascular disease hypertension, nervous system dysfunction and reproductive disorders. Most of the epidemiological studies conducted so far have focused on the cancer risk in this industry. Detection of hazardous environmental agents and measurement of early biological responses in individuals exposed to such agents are among the major goals of today's preventive toxicology. Cytogenetic techniques used to study chromosomes of somatic cells offer, in theory, an outstanding possibility of detecting genetic damage induced by exogenous agents. Increasing number of substances used in the synthesis of polymers and in the manufacture of goods are being added to the environment. The rubber industry is known to use a large and expanding array of chemicals some of which are experimentally proven mutagens and carcinogens. Rubber products vulcanized at high temperature and pressure emit biologically active chemical agents. Several of them are known to be suspected mutagens and carcinogens (Falck 1983). In epidemiological studies it has been proven that workers in rubber industry may have an increased cancer risk (Lemen et al. 1990; Matanoski et al 1990). Derivatives of styrene, butadiene and rubber are carcinogenic in animal studies (Huff et al. 1985; IPCS 1983) and mutagenic *in vitro* and *in vivo* systems (IPCS 1983; Cunningham et al. 1986; Geravasi et al. 1985; Rosenthal 1985; Tice et al. 1987). They induced sister-chromatid exchanges (SCE) and chromosomal aberrations whereas several studies showed an increase in the

frequencies of CA of workers from rubber industry (Anderson et al. 1980; Cammuri et al. 1983; Sorsa et al. 1983) others did not find any increase (Degrassi et al. 1984). To resolve this confusion, workers from a rubber tyre industry were investigated for cytogenetic damage, haematological and biochemical changes due to work place pollutants.

Materials and Methods:

Selection and grouping of the participants

The present investigation included 15 individual who all worked in tyre industry and were mainly exposed to a variety of chemicals.

Data recording

The workers were selected based on the following criteria

1. They should have been employed full time, working 7 to 9 hours per day with a work experience of not less than five years.
2. They should have no previous exposure to any other pollutants.
3. They should not be chronically suffering from any other major complications.
4. They should not be smokers.

Fifteen healthy volunteers in the age group of 20-50 years not exposed to any toxic industrial pollutants were selected and this served as control.

Collection of blood samples and separation of serum

Five ml of venous blood was collected from each participant and transferred into EDTA coated tubes (Sharma and Awasthi, 2001). Eight ml of venous blood was collected from both the experimental and control group after overnight fasting and transferred to sterilized vial. The blood was then centrifuged at 3,500rpm for half an hour to obtain the serum.

Analysis of hematological parameters

The EDTA blood samples were used for analyzing Haemoglobin (Hb), Red blood cell count, (RBC) white blood cell(WBC) count and Erythrocyte sedimentation Rate(ESR). Haemoglobin content was estimated by cyanmethaemoglobin method of wintrobe et al (1965). The total red blood cell count was determined by haemocytometer method of wintrobe et al (1965). Total leucocytes count was determined by Truck's fluid method Raghuramulu et. al (1983) and the ESR was estimated by westergren method

Analysis of other selected bio-chemical parameters

The triglyceride, total cholesterol, HDL cholesterol, ALT, AST, and LDH was estimated by kit method and Estimation of amylase, zinc, lead were determined by atomic absorption Spectrophotometric method.

Assay of Enzymic antioxidants

Antioxidants protect the body from damage caused by free radicals. Many experts believe that this damage is a factor in the development of blood vessel disease, cancer and other condition (Husney 2005) the superoxidedismutase activity was determined by the method Kakkar et al (1984), the activity of catalase was measured by Luck (1974), Glutathione peroxidase was estimated by Rotruck et al. (1973)

Screening for chromosomal aberration in peripheral lymphocytes of rubber industry workers

Heparinised blood samples were collected under aseptic condition from control group (n=5) rubber industry workers (n=5), the blood samples were used for the lymphocytes culture to study frequencies of chromosomal aberration in the selected subjects.

Statistical Analysis

Analysis of variance was followed to compare the results obtained for various estimations carried out in experimental and control subjects.

Results and Discussion

The results obtained for the selected haematological parameters in rubber industry workers are shown in Table 1. It is evident that there was a significantly decreased level of Hb, RBC, WBC and increased level of ESR obtained in the rubber industry workers might be due to the exposure of these participants to various solvents like benzene, 2-methox ethanol and nitrosamine used in the processing of rubber. Shih et al (2003) recorded decreased levels of HB, RBC in workers exposed to 2-methoxy ethanol. As shown in Table 2, it is evident that there was a significantly increase ($p < 0.05$) in the level of triglycerides, total cholesterol, VLDL-C, HDL-C and LDL-C in the experimental participants when compared to those of control participants. As may be seen in Table 3, the levels of serum enzymes ALT, AST, LDH and amylase were found to be increased in rubber industry workers when compared to those of respective control. High levels of two key liver enzymes ALT and AST may be sign of liver damage. Several HIV patients can cause elevated liver enzymes in HIV patients (Highleyman 2003). The rubber industry workers were exposed to rubber dust and rubber fumes measured as cyclohexane soluble matter. Hence the increased level of ALT, AST, LDH and amylase in serum might be due to their exposure to rubber dust and fumes. The levels of serum protein in experimental group participants decreased significantly ($p < 0.05$) on comparison with the control subjects. In Table 4, it can be inferred that the levels of serum protein in experimental group participants decreased significantly $P < 0.05$ on comparison with the control subjects. Diminished serum protein level might be associated with excessive loss due to nephrosis or to reduced protein synthesis due to cirrhosis (Sharma 1989)

The loss in serum protein in experimental group participants might be the consequence of liver and kidney damage as a result of exposure to toxicants. The high level of serum zinc in rubber industry workers might be due to the usage of zinc and other heavy metals for the purpose of coating. Excess zinc and other heavy metals might cause neurodegenerative diseases and in due course toxic responses in the body (Ravid and Rao 2006).

As shown in Table 5, the serum levels of the three enzymic antioxidants namely SOD, CAT, GPX were

reduced significantly in experimental group participants when compared to those of control subjects. Heavy metal stress results in the production of O_2 , H_2O_2 , and OH which affect various cellular processes, mostly the functioning of membrane systems. Cells are normally protected against free radicals by the intricate antioxidant systems. The concentration of antioxidant enzymes SOD, CAT, GPX were found to be decreased in both digestive gland and kidney of the gastropod, *Achatina Fulica* treated with $CdCl_2$ and $ZnSO_4$ (Chandran et al 2005). The data given in Table 6 represents the results of the chromosomal aberration studies in the selected rubber industry workers. The incidence of chromosomal aberration was more with the increasing concentration of chemical pollutants. Some of the toxic chemical function as mutagens and cause not only aberration but also damage DNA and cell (Ouseph et al 2000). The mitotic index, chromosomal aberration, sister chromatid exchange and satellite associated were analysed in rubber type industry workers.

Conclusion

From the results obtained in the preset study, it can be concluded that the occupational exposure to different chemical compounds, fumes and gases in the rubber industry causes changes in the hematological and biochemical parameters in the industry workers. The genotoxic study showed that the rubber industry workers are at a high risk of developing cancer.

Acknowledgements

We would like to thank the employers and employees in the rubber manufacturing industry for the close cooperation in this study. We also would like to acknowledge Mrs. Bhuvaneshwari Reader, Dept of Biochemistry and Biotechnology, Avinasilingam University, Coimbatore.

Table 1: Mean level of haemoglobin red blood cell count, white blood cell count and Erythrocyte sedimentation rate for the selected rubber industry workers.

S.No	Parameters	Control group (n=15)	Experimental group(n=15)
1	Haemoglobin (g/dl)	15.9	10.0
2	Red blood cell count($\times 10^6 \text{mm}^3$)	5.3	2.7
3	White blood cell count ($\times 10^6 \text{mm}^3$)	7515	3450
4	Erthrocyte sedimentation rate (mm/hr)	7.9	25.5

Table 2: Mean levels of Triglycerides, total cholesterol, HDL – C, LDL – C and VLDL-C for the selected rubber industry workers.

S.No	Parameters (mg/dl)	Control group (n=15)	Experimental
------	--------------------	----------------------	--------------

			group(n=15)
1.	Triglycerides	113.0	170.7
2	total cholesterol	166.3	284.4
3	HDL – C	45.8	58.1
4	LDL – C	93.0	202.4
5	VLDL- C	23.4	34.1

Table 3: Mean levels of selected serum enzymes in rubber industry workers.

S.No	Parameters	Control group (n=15)	Experimental group(n=15)
1.	ALT (IU/L)	17.3	33.3
2	AST(IU/L)	22.8	34.3
3	LDH(U/L)	152.5	315.6
4	AMYLASE (U/dl)	95.4	287.1

Table 4: Mean levels of Serum Protein ,Lead and Zinc in rubber industry workers.

S.No	Parameters	Control group (n=15)	Experimental group(n=15)
1	Protein (g/dl)	6.5	4.9
2	Lead (µg/dl)	24.8	58.5
3	Zinc(µg/dl)	88.8	167.3

Table 5: Mean levels of Serum Superoxide dismutase, Catalase, Glutathione peroxidase in rubber industry workers.

S.No	Parameters	Control group (n=15)	Experimental group(n=15)
1	Superoxide dismutase	4.5	3.3
2	Catalase	11.0	4.8
3	Glutathione peroxidase	21.5	17.1

Table 6: Mean frequency of Chromosomal aberration in rubber industry workers.

Group	No of chromosome	No of chromosomes with aberration	No of cells analysed	Chromosomal aberration /cell	Mean ± SD
Control					
1	3818	78	83	0.93	0.932±
2	4002	85	87	0.97	
3	3956	81	86	0.94	

4	4232	89	92	0.96	0.043
5	3266	61	71	0.86	
Experimental Group					
1	2990	161	65	2.47	2.269±
2	3358	178	73	2.43	
3	2806	141	61	2.31	
4	3772	163	82	1.98	0.203
5	3266	153	71	2.15	

References

- Buccolo G. (1973). Quantitative determination of serum triglycerides by use of enzymes. *Clinic. Chem*, **19(5)**:476-482.
- Chandran, R., Sivakumar, A.A., Mohandass, S. and Aruchami, M (2005). *Comparative Biochemistry and Physiology part C: Toxicology and pharmacology*, **140**,422-426.
- Cody, R.R. and Strawderman, W.W. and Kipen, H.(1993) Hematologic effect of benzene, *J. J. Occup. Med.***35(8)**:776-782.
- Daice, J.V. and Lewis, S.M. (1984) *Practical haematology* , Sixth edition, Churchill living stone, London,**22** 117.
- Dhanapakiam, P. and Ramasamy, V.K. (2001) Toxic effects of cooper and zinc mixtures on some haematological and biochemical parameters in common carp, *Cyprinus carpio(Linn)*, *JEnviron. Bil.* **22(2)**:105-111
- Hilt, B;Romayhr, T.Q.(1999), Morbidity from is chemic heart disease in workers at a stainless stel welding factory, *Norsk Epidemiology***9(1)**:21-26.
- Lumes,(1999) *The rubber industry and extended producers responsibility frame work Chapter I,3-5.*
- Malcom, L., Bigden, M.D.(1999) Clinical utility of the Erythrocyte sedimentation rate, *Journal of Ameriacal Family physicinal*,601,No.5.
- Mathad,P., Angadi, S.D and Mathad, R.D.(2004) Short and long term effects of exposure of microalgae to aheavy metal stress, *Asian Jr. of microbial. Biotech. Environ. Sci.* **6,(1)**:99-106.
- Murray, R.(1984) *Alanine aminotransferase Kalpana Clinical chemistry. TheC.V. Mosby co.st Louis Toronto. Princeton*, 1088-1090.
- Murray, R. (1984) *Aspartate aminotransferase Kalpana Clinical chemistry. TheC.V. co.st Louis Toronto. Princeton*, 112-116.
- Naito, H.K., Kalpana, A(1984) High density lipoprotein(HDL)Cholesterol, *clin chem. The C.V. co.st Louis Toronto. Princeton*, 1207-1213 and 437.
- Ouseph, A., Sundarsanam, D., Nainar, A.A., and Gandheeswari, P. (2000). Frequency of chromosomal aberration in fish inhibiting pollutedecosstem, *Poll, Res.***19 (1)**:123-128.

- Raghuramulu, N., Madharan Nair, K. and Kalyansundaram, S. (1983) A manual of laboratory techniques, National institute of Nutrition, Hyderabad, Silver prints, Hyderabad, 78: 257-258.
- Ravid, B.R., Rao, K.S. (2006) Role of metals in neuronal apoptosis: Challenges associated with neurodegeneration. *Curr . Alzheimer Res*: **3**:311-326.
- Schulte, P.M., Glemet, H.C., Poers, A.D. (2000). Adaptive variation in lactate dehydrogenase expression, Role of a stress-responsive regulatory element, *Proc National Academic Science U.S.A.* 97(12):6597-6602.
- Sharma, S.D. (1989) Haemachemical adversities in *clarias batrachus* induced by lithium, *Ind. J. Environ HLTH*, **31**, (4), 354-357.
- Sharma, M.L and Awasti A.K (2001). Haematological and biochemical characteristics of *Heteropneustes Fossilis* Under the stress of congo red (diphenyl disazo biophthionic acid), *Toxicology letters*, 14, 237-241.
- Shih, T.S., Hsieh, A.T., Chen, Y.H., Liao, G.D., Chen, C.Y. Chou. J.S. and Liou, S.H. (2003) Follow up study of haematological effects in workers exposed to 2-methoxy ethanol, *occup.Environ. Med.* 60, 130-135.
- Straif, K., Weiland, S.K., Bungers, M., Holthenrich, D.M., Taeger. D., Yi, S., Keil, U. (2000) Exposure to high concentrations of nitrosamines and cancer mortality among a cohort of rubber workers, *Occu. U. Environ. Med* **57**, 180-18.
- Varley., Horald., Alan., H., Gowenlock and Bell M. (1991). *Practical clinical biochemistry*, Vol.1, Fifth edition, CBC publishers and distributors, New Delhi, **720**:671-677.
- Vermeulen, Ronsson, Bo.A.G, Lindh, C.H., Kromhout, H. (2005). Biological monitoring of carbon disulphide and phthalate exposure in the contemporary rubber industry, *Int Arch Occup Environ Health*, 78:663-669.
- Wang-X:R. and Christiani, D.C., Tan ,X. (2002). Occupational lung disease in China, *Occup. Med.* **17**:365-376.
- Wijngaarden, E.V. and Stewart, P.A. (2003). Critical literature review of determinants and levels of occupational benzene exposure for incited states community based case-control studies, *Applied occupational and Environmental Hygiene*, **18**: 678-693.
- Win robe, M.M., Lee, G.R., Boggs, D.R., Bothell, T.C., Forester, J., Athens, J.W., and Lukens J.N (1965) *Clinical Haematology*, Eighth edition, Lea and Febiger, Philadelphia, 30-35:42-46.