Assessment of Genotoxicity in Occupationally Exposed Industrial Workers of Visakhapatnam City.

Human Genetics

KEYWORDS : Genotoxicity, Industrial workers, Micronuclei, Genomic Damage, Industrial Pollution.

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ABSTRACT

Introduction: *Biomonitoring of human genotoxicity induced by complex occupational or environmental exposure to genotoxic agents is highly essential. Genotoxicological biomonitoring in human population is a useful tool to estimate the genetic risk. This genetic risk was estimated by comet assay and micronuclei assay.*

Materials and methods: *Sample size of the present study includes 20 individuals of who are occupationally exposed to industrial pollution in the region of Parawada, Visakhapatnam District, Andhra Pradesh, India. And 20 healthy normal individuals of the same age and sex from the same region who are not exposed to industrial pollution as the controls with age above 20 during the period march 2015 to September 2015. 5ml of blood sample collected from both study and control groups for genotoxicity study, by using comet assay and micronuclei assay.*

Results: *The comet tail length mean value of exposed people to industrial pollution was high when compared with the nonexposed people to industrial pollution. Significant increase in micronuclei and binucleated cells was observed in people exposed to industrial pollution compared to nonexposed controls, whereas the mean frequency of single nucleated cells in nonexposed controls was more when compared with the exposed individuals.*

Conclusion: *The study correlates with the findings of earlier studies carried out in India and other countries. It emphasizes the workers exposed to industrial pollution presented more genomic damages than non exposed people either evaluated by comet assay or micronuclei assay.*

Introduction

Environmental pollutants are known carcinogens and mutagens and testing for chromosomal aberrations (CA) and DNA damage is the most common technique to assess their impact (Maronpot, 1991; Albertini et al., 2000; Natarajan, 2005) The CA assay is considered to be a strong classical cytogenetic method for testing genotoxicity and can be used as a validation test for the results of Comet assay (Anwar, 1994; Testa et al., 2002). Such studies are now common in developed countries but much more remains to be done in developing countries (Anwar, 1992).

The industries produce large quantities of effluents, which when released untreated into environment, leads to air, soil and water pollution. Exposure to solvents is one of the highest potential risks for million of industrial workers in the world. It can generate substantial environmental pollution leading to outbreaks of public health problems (Torres et al., 2008). Waste water from different sources was found to have cytotoxic effect with different concentrations. Chronic occupational exposure to printing dyes may lead to an increased risk of genetic damage (Aksoy et al., 2006).

Genomic damage is probably the most important fundamental cause of developmental and degenerative diseases. Genomic damage is produced by environmental exposure to genotoxins and life style factors like alcohol, smoking, drugs and stress (Ozkul et al., 1997; Speit et al., 2006; Chen et al., 2006). Hence, biomonitoring of human genotoxicity induced by complex occupational or environmental exposure to genotoxic agents is highly essential. Genotoxicological biomonitoring in human population is a useful tool to estimate the genetic risk from an integrated exposure to complex mixture of chemicals. The use of biomarkers associated with these events provides useful tools for the early detection of disease related changes and micronucleus assay has been found to be an excellent tool to serve as a genotoxicological biomarker. (Sarto et al., 1990; Kyrtopoulos et al., 2006).

The Comet assay also known as Single Cell Gel Electrophoresis, especially its alkaline version, allows sensitive detection of DNA damage in various settings (Cotelle et al., 1999; Tice et al., 2000; Møller et al., 2000; Wong et al., 2005; Møller, 2006; Valverde et al., 2009; Piperakis, 2009).Comet assay can be used to examine the impact of the industrial waste on the human population directly. Micronuclei are small chromatin bodies that appear in the cytoplasm by the condensation of acentric chromosome fragments or by whole chromosomes, lagging behind the cell division. Thus, it is the biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells, since they are easily detected in interphase cells. In this way, the analysis of MN in epithelial cells has shown to be a sensitive method for monitoring genetic damage in human populations (Sarto et al., 1990; Karahalil et al., 1999; Majer et al., 2001).

There is no sufficient information for the assessment of genotoxicity concerning the occupational exposure to industries. Considering these, the present study aimed to investigate the genotoxic effects in occupationally exposed industrial workers of Visakhapatnam city by using comet assay and MN test.

Methods

Sample size of the present study includes 20 individuals of who are occupationally exposed to industrial pollution in the region of Parawada, Visakhapatnam District, Andhra Pradesh, India. And 20 healthy normal individuals of the same age and sex from the same region who are not exposed to industrial pollution as the controls with age above 20 during the period march 2015 to September 2015.5ml of blood sample collected from both study and control groups for genotoxicity study.

Comet Assay

In vitro test was performed according to the method of Singh et al., 1988 with small modifications. 1×10^6 lymphocytes were suspended in 0.5% low melting point agarose and sandwiched between a layer of 0.6% normal melting point agarose and a top layer of 0.5% low melting point agarose on fully frosted slides. During polymerization of each gel layer the slides were kept on ice. After solidification of the 0.6% agarose layer the slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 1% Triton X-100 and 10% DMSO) at 4°C. After 1 hour slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, pH 10) for 20 min at room temperature to allow for DNA unwinding. Electrophoresis was conducted in a horizontal electrophoresis platform in fresh, chilled electrophoresis buffer for 20 min at 300 mA and 19 V. The slides were neutralized with Tris-HCl buffer, pH 7.5, three times for 5 min and stained with 10% ethidium bromide for 10 min. Each slide was analyzed using a fluorescence microscope. For each subject 50 cells were analyzed with an automatic digital analysis system, determining tail length.

Micronuclei Assay
The in vitro test was performed according to the method of Fenech and Morley, 1986 with small modifications. Add 0.5 ml of heparinized whole blood in 5 ml of RPMI 1640 supplemented with 20% fetal bovine serum, 50 ml L-glutamine, antibiotics, and 1.0% phytohemagglutinin. Incubated the tubes at 37°C for 44 hours, later add 37.5 µl of cytochalasin-B to each tube. At 72 hours, make a gentle fixation of methanol/acetic acid (3:1). Cell suspensions are dropped from each tube onto five clean and cold slides from a 2-3 cm distance and a slide inclination of 45°. Slides are allowed to air dry and incubated at 60°C overnight and stained with Giemsa stain. The number of multinucleate cells and number of bi nucleate cells are counted to calculate the mean percent (%) of cells at multinucleate stage.

Results
Table 1: Mean comet tail length (µm) in exposed and nonexposed people to industrial pollution.

Groups	Tail length (µm)
	Mean ± Standard Deviation
Exposed(20)	
Males(10)	4.46 ± 1.54
Females(10)	3.46±1.07
Total	3.96 ±1.42
Non-exposed(20)	
Males(10)	2.96± 1.0
Females(10)	2.54± 0.53
Total	2.75 ± 0.83

Table 1 represents the mean values of comet tail length in exposed and non exposed peoples to industrial pollution. The mean values of males and females in exposed people to industrial pollution were 4.46 and 3.46 respectively. The mean value of nonexposed males to industrial pollution was 2.96 and females was 2.54. The total mean value of exposed people to industrial pollution was 3.96, where as the total mean value of nonexposed people to industrial pollution was 2.75. From the above table it was inferred, that the comet tail length mean value of exposed people to industrial pollution was high when compared with the nonexposed people to industrial pollution.

Table 2: Mean frequency of micronuclei in exposed and non exposed people to industrial pollution.

Groups		Parameters	
	Micronuclei (±)SD	Binucleated (±) SD	Single nucleated (±) SD
Exposed			
Males(10)	2.8 ± 1.3	4.8 ± 2.03	92.4 ± 2.69
Females(10)	2.4 ± 1.2	4.2 ± 1.88	93.4 ± 2.05
Total	2.6 ± 1.2	4.5 ± 1.9	92.9 ± 2.44
Non-exposed			
Males(10)	0	4.1 ± 1.86	95.90 ± 1.86
Females(10)	0	3.7 ± 1.84	96.30 ± 1.84
Total	0	3.9 ± 1.86	96.1 ± 1.86

Table 2 represents the mean frequency of micronuclei in exposed and nonexposed people to industrial pollution. The mean frequencies of micronuclei in males and females exposed to industrial pollution were 2.8 and 2.4 respectively and micronuclei were completely absent in nonexposed people to industrial pollution.

The mean frequencies of binucleated cells in males and females exposed to industrial pollution were 4.8 and 4.2 respectively, where as the mean frequency of nonexposed males was 4.1 and females was 3.7.

The mean frequencies of single nucleated cells in males and females exposed to industrial pollution were 92.4 and 93.4 respectively, where as the mean frequency of nonexposed males was 95.9 and females was 96.3.

The total mean frequencies of micronuclei and binucleated cells of people exposed to industrial pollution were 2.6 and 4.5 respectively, where as the total mean frequency of single nucleated cells exposed to industrial pollution was 92.9.

The total mean frequencies of binucleated cells and single nucleated cells in people nonexposed to industrial pollution were 3.9 and 96.1 respectively, where as the micronuclei were completely absent.

It was evident from the above table, significant increase in micronuclei and binucleated cells was observed in people exposed to industrial pollution compared to nonexposed controls, where as the mean frequency of single nucleated cells in nonexposed controls was more when compared with the exposed individuals.

Discussion
Mutagenesis involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to the development of pernicious illnesses, many times through mechanisms that involve genetical changes. In order to evaluate the possible impact of environmental and occupational exposition on health, it is essential to identify the effects of exposure. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposition and to monitor populations that are excessively exposed (Maluf and Erdtmann, 2000). In this regard a constant exposure of humans to toxic compounds in the workplace may lead to mutagenic/carcinogenic effects.

In the present study the comet tail length mean value of exposed people to industrial pollution was high when compared with the nonexposed people to industrial pollution. Various studies have demonstrated the effect of textile effluents on different animal models including Sumathi and Manju, 2001. Many studies have revealed the deleterious effects of textile dyes on the human health. Similarly, Singh and Randhawa, 2004 found a significant difference (Mann Whitney, p<0.05) in the CTL values among exposed and control subjects. Kwon et al., 2008 assessed the mutagenic activity of river water from a river near textile industry on human lymphocytes and found similar results showing increased mean comet tail lengths. Gemitha et al., 2013 found an increase in DNA damage in workers from rubber industry was also reported by Sorsa et al., 1983 and Sasiadek, 1992. Manikantan et al., 2009 found Photocopy machine workers shows significant effects on DNA damage when compared to nonexposures.

Significant increase in micronuclei and binucleated cells was observed in people exposed to industrial pollution compared to nonexposed controls, where as the mean frequency of single nucleated cells in nonexposed controls was more when compared with the exposed individuals in the present study. Our findings are in agreement with those of the Sram et al., 1998, who detected a significant increase in the frequency of micronuclei in the lymphocytes of exposed workers.

Of the various confounding factors studied, duration of exposure showed significant effects and it has also shows a positive effect on DNA. Similarly for example, workers of phosphate fertilizer factory, paint manufacture industry, outdoor painters, cement workers, aluminium sulphate worker, those exposed to sulphur dioxide and environmental factors and low dose ionizing radiation showed increased frequency of genetic damage with increase in duration of work suggesting a cumulative genotoxic effect (IARC, 1989; Albin et al., 1990; Diaz et al., 1990; Ajoy et al., 1990; Yadav and Seth, 1998; Yadav and Thakur, 2000; Balachandar et al., 2008)

Occupational exposure to these xenobiotics may result in covalent binding of their molecules to DNA, which leads to chromosome alterations and may be an initial event in the process of chemical carcinogenesis (Miller, 1978; Keith and Dirheimer, 1995; Eastham et al., 2001). The present findings highlight the importance of using comet assay and MN test to detect DNA damage and genotoxic effect of various confounding factors, since this information provides an increased degree of identification for the positive response.

In conclusion biomonitoring studies of workers exposed to industries are rather specific because each population has a different life style factors but same occupation in different areas under different climatic and environmental conditions and are exposed to indistinguishable of mutagen. This could explain why some studies find an increase of genetic damage in general populations. Another explanation for the genotoxic damage observed can be the lack of protective measures taken by the workers. Therefore, there is a need to educate those who work with industries about the potential hazard of occupational exposure and the importance of using protective measures.

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