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Genotoxic Profile of Motor Garage Workers

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Abstract: Problem statement: One way to study the effects on an exposed population is to conduct monitoring studies, using relevant biological parameters with a short term manifestation, such as cytogenetic analysis express the damages to the DNA or to the chromosomes resulting from exposure can be identified. The present study was thus designed to elucidate the effect motor garage environment on the workers. **Approach:** Peripheral blood culture was performed with blood collected from workers of Motor garages in Ahmadabad, situated at central Gujarat region. Our objective was to establish the relationship of the environment induced Micro Nucleus (MN) and Chromosomal Aberrations (CA) with non-occupational factors, such as the smoking and drinking habits since quality of life and nutritional status are the key factors that influence toxicity profile. **Results:** On comparing the smokers of the exposed group with that of smokers of control group the structural chromosomal aberration and micronucleus induction frequency was significant high ($p<0.02$) in the exposed group. Exposed individual with non smoking habit also exhibited remarkable increase in the CA and MN values with a significant increase ($p<0.02$) when compared with control. Considering the drinking habit of the exposed individual to that of the control, the exposed group showed significant increase ($p<0.05$) in the CA as well as MN. **Conclusion:** This indicates that occupational exposure to the many chemicals of the garage environment can have Genotoxic effects and these effects increases with the increase in period of exposure.

Key words: Micronucleus induction, chromosomal aberrations, peripheral blood culture, genotoxic effects, exposed group, Micro Nucleus (MN), motor garage

INTRODUCTION

Knowledge of human health risks related to environmental exposure to hazardous chemical agents is a current concern (Franco *et al*., 2008; Akan *et al*., 2009). Populations of industrial areas are intensely exposed to chemical substances that can cause mutations, cancer and congenital defects (Hirvonen, 1995). The majority of situations involve exposures to low doses for long periods, which in many cases involve the individual's entire lifetime and result in small increments in health risks (Franco *et al*., 2008). Occupational agents can induce several types of cancer, such as urinary tract, skin, larynx and pancreas cancers and leukemia (Santos-Mello and Silva, 1996). One way to study the effects on an exposed population is to conduct monitoring studies, using relevant biological parameters with a short term manifestation, such as cytogenetic analysis, by which damages to the DNA or to the chromosomes resulting from exposure can be identified (Pant and Rao, 2010). The obtained information can be used as an early warning about the potential risk of health problems developing in the long run.

Exposure to a wide range of industrial chemicals including heavy metals, contained in brake fluids, degreasers, detergents, lubricants, metal cleaners, paints, fuel, solvents, resulting in various forms of chronic poisoning.

Air quality measurements in vehicle garages indicate that high pollutant concentrations caused by car-induced emissions may occur in the motor garage (Karahalil *et al*., 1998). Benzene dusts, NO_2, PAH and CO are the main pollutants present in the motor garage. Most garages generate hazardous waste, waste water, air emissions and pollutants from such services as fluid replacement or operations like washing or painting parts. There are several types of solid waste. Lead acid is present in the car batteries which is big pollutant. Along with that the paints which contains many aromatic hydrocarbon also contributes in the pollutant list (Martino-Roth *et al*., 2002).

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To augment the quality of the life of the intimate workers and workers related to such small scale industry we carried out this pilot based study. Our objective was to evaluate by MNT and CA the level of damage and to establish the relationship of the Micro Nucleus (MN) frequency and (CA) with non-occupational factors, such as the smoking and drinking habits.

MATERIALS AND METHODS

Subjects: The present investigation was divided into two groups; test and control. Test group included 5 individuals working in an automotive workshop situated at Narol area, Ahmedabad, Gujarat. Control group consisted 5 age matched individuals not exposed to automotive pollutants, mainly office workers. The health and occupational histories of the subjects and controls with respect to age, sex, duration of exposure, socioeconomic condition, nutritional status, smoking and drinking habits were collected. None of the individual had been exposed to x-rays or had taken antibiotics for three months prior to sampling. The worker do all kind of jobs including spray painting, carving, engine fitting. Many a times without using any protective device. These workers were engaged in work for more than 8 hrs per day with a minimum 5 yrs of exposure duration. Workshop owner and worker were educated about objective of the study and duly signed consent forms were obtained from all workers.

Blood collection: Peripheral blood of 5 individuals within the age group 20-30 yrs was collected in plain screw capped tube coated with heparin. The blood was taken to the laboratory within 2 hrs of collection. Short term lymphocyte cultures were established from heparinised blood according to the method by Hungerford (1965).

Experimental groups: There were two groups formed test (from motor garage) and controls (normal healthy individuals). All these groups were then further divided into subgroups on the basis of smoking and drinking habits.

Preparation of culture media: RPMI 1640 powder was obtained from Himedia Pvt Ltd, Mumbai, was dissolved in 800 mL autoclaved D/W. To this 2 gms NaHCO3, 100 mg penicillin (100,000 units) and 100 mg streptomycin were added and dissolved. Then the pH of solution was adjusted to 7.3. Then the media was filtered by negative pressure vacuum filteration followed by packing and labeling of the media bottles. The procedures from vacuum filtration onwards were carried out in laminar air flow. Prepared media was stored at 4 C after labeling.

Peripheral Blood Lymphocyte Culture (PBLC): Peripheral blood lymphocyte culture was carried out by the standard procedure by Hungerford (1965), with slight modification. Blood was collected in heparinized syringe and was properly mixed to avoid any clot formations. 7 mL RPM-I1640 media containing 10% FCS, Penicillin and Streptomycin was used to set up the culture in duplicates. 0.1 mL of Phytohaemagluttinin (PHA) (1 mg mL^{-1}) and 9 drops of blood were added to each tube, mixed properly and kept in incubator at 37 C after closing it tightly. This day was counted as day zero. At 69th hr the 20 µL colchicine (1 mg mL^{-1}) was added to all the tubes, so as to arrest the cells at metaphase.

Harvesting: After 30 min incubation with colchicines, the cultures were taken out of the incubator and cells were pelleted down by centrifuging at 2000 rpm for 12 minutes. Then 5 mL prewarmed hypotonic solution (0.56%) was added to the pellet and flushed gently with dropper. The tubes were then incubated at 37°C in water bath for 20 min and then 2 mL chilled fixative (1:3 acetomethanol) was added, flushed properly and centrifuged at 2000r pm for 20 min. The pellet was washed twice with fixative and finally the pellet was suspended in 0.5 mL of chilled freshly prepared fixative.

Preparation of slides: Frosted slides were first soaked in chromic acid overnight and then kept under running tap water in horizontal coupling jar. After washing the slides with D/W, they were then transferred to a beaker filled with chilled D/W. then 3-4 drops of suspension was added to the slides and flamed on the spirit lamp. Slides were then observed under the microscope after air drying.

Giemsa staining: Slides were dipped in 2% Giemsa for 3 min and then carefully rinsed with tap water in coupling jar. After air drying, slides were observed under the microscope for well spread metaphase plates to screen for chromosomal aberrations including structural aberrations and numerical aberration (viz chromosomal gaps, breaks, aneuploidy).

Structural aberrations analysis: Giemsa stained metaphase plates were scored for the chromosomal aberrations. The aberrations can be divided into two groups chromosomal and chromatids aberrations. Chromosome aberrations are one where both chromatids are affected at the same loci while in

chromatids or mis alignment of proximal and distal parts of chromatids are observed with unstained region then it was scored for gaps/breaks. Chromatids gaps includes unstained region not larger than the diameter of chromatids and breaks includes unstained region more than the diameter of chromatid. Similiary chromosome aberrations were also divided into two types, based on the same criteria.

Binucleate induction and Micro Nucleus (MN) analysis: Binucleate induction and micronucleus analysis was done following the method of Fenech (2000) with slight modifications. Cytokinesis was inhibited by the addition of cytochalasin B to the cultures. With this technique it is possible to observe the products of single cell division and to assess the rate of micronucleus formation in the cell division. Whole blood cultures were established by standard procedure as described earlier. After 72 hrs of incubation, cytochalisine B was added to a final concentration of 30nµL/mL. At 96hrs the culture were centrifuged and 5 mL pre-warmed (37°C) 0.075M KCl was added to the tube and kept for 25 min at 37°C. The cells were then fixed in fresh chilled fixative and kept in ice bath for 20 min. The tubes were centrifuged and cells were given one wash with fixative. The total period of fixative was about 2 h. The slides were stained in 4% giemsa. Binucleates were scanned for micronucleus under high power objective for each individual at least 500 binucleates were scanned.

Nuclear Division Index (NDI): NDI is often calculated according to the method of (Eastmond and Tucker, 1989). Five hundred viable cells are scored to determine the frequency of cells with 1, 2, 3 or 4 nuclei and calculate the NDI using the formula:

NDI= M1+2(M2) +3(M3) +4(M4)/N

where, 1-M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored. The NDI and the proportion of binucleated cells are useful parameters for comparing the mitogenic response of lymphocytes and cytostatic effects of agents examined in the assay.

RESULTS

Mitotic index: The result obtained for the mitotic index and the structural aberration studies between the exposed motor garage workers and normal non exposed individual control is given in the table below (Fig. 1). The mean MI in exposed individual was 6.02+0.6265 which was slight higher when compared to control individual which showed a MI of 3.70+0.254. The value of mitotic index for the other group did not showed any significant increase when compared to that of control.

The Mitotic Index of exposed individual is elevated in comparison to controls. The MI is seen high in the workers having the drinking and smoking habit.

Chromosomal aberrations: Increase in the frequency of structural aberration of exposed individual when compared with the control. The mean SCA in the exposed individual was 13.16+5.928 while in the non exposed control it was 3.0+1.643. On comparing the smokers of the exposed group with that of smokers of control group the structural chromosomal aberration was significant high ($p<0.02$) in the exposed group. Exposed individual with non smoking habit also exhibited remarkable increase in the SCA value with a significant increase ($p<0.02$) when compared with control. Considering the drinking habit of the exposed individual to that of the control, the exposed group showed significant increase ($p<0.05$) in the SCA. There was increase in the SCA value for the non drinking exposed group when compared to control but there was not statistically significant (Fig. 2).

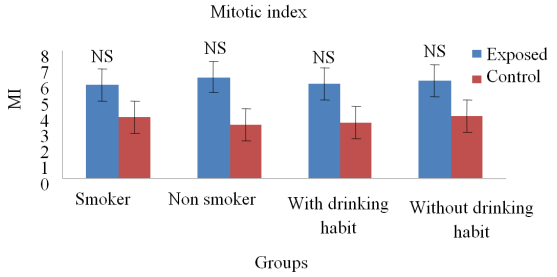

Fig. 1: The mitotic index of the exposed individual compared to controls, Non Significant (NS)

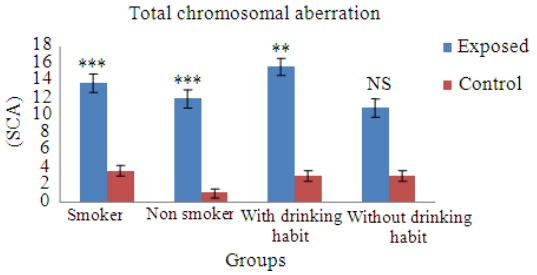

Fig. 2: Increase in the total chromosomal aberration is seen in the exposed group in comparison to non exposed control group, *** Significant at $p<0.02$, ** significant at $p<0.05$, Non Significant (NS) when compared to respective control

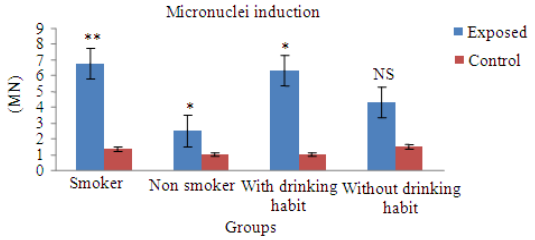

Fig. 3: Micronuclei induction of individual exposed to pollutants in motor garage compared to normal control individuals **Significant p<0.05, *significant p<0.01, Non Significant (NS)

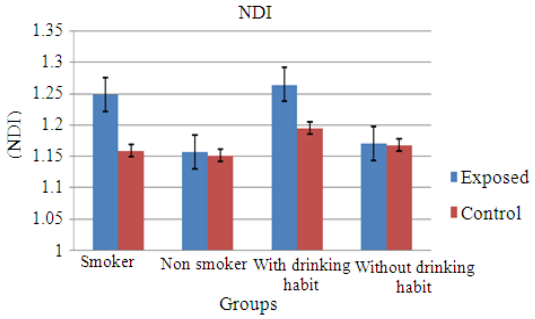

Fig. 4: Exposed individual show increase in the NDI compared to non exposed control, Non Significant (NS)

Micronuclei: The results obtained for the stimulation of micronuclei in individual with exposure to motor garage pollution in comparison to normal control individual is in Fig 3. A remarkable increase ($p<0.01$) in the frequency of MN induction was observed in comparison to the control group. On comparing the smokers of the exposed group with that of smokers of control group the induction of MN was significantly high ($p<0.05$) in exposed group. Exposed individuals with no smoking habits also exhibited a remarkable increase ($p<0.01$) in the MN frequency compared to non smoker controls. Considering the drinking habits, exposed group showed a significant rise ($p<0.01$) in the induction MN to control group with drinking habit. However comparing the exposed and control group, without drinking habit, did not show any significant rise in the frequency of MN induction.

It is seen from the graph that the frequency of MN formation is higher in exposed workers compared to controls. Smoking and drinking habit also shows some correlation with the frequency of MN formation.

NDI: The result obtained from the NDI of the present study doesn't show any statistically significant increased in the exposed individual when compared to the non exposed control (Fig 4). The NDI for the smoking habit individual showed increased in NDI index when compared to their control, same was the observation in the case of the individual wit drinking habit. But there was no significant increase seen in the non smoker of the exposed group when compared with control. The exposed individual also showed the same result as shown by the non smoker group.

DISCUSSION

This result is in accord with the findings of Santos-Mello and Silva (1996), who investigated chromosome alterations in car painters from the city of Brasilia, discovering a significant increase in chromosome and/or Chromatid anomalies (96%) in the risk group, as compared to the control group (55%). They found a deletion rate of 60% in the painters and of 20% in the controls, a proportion similar to the one found in this study.

Car painters are exposed to the action of a great number of chemicals, such as solvents whose foundation is ketone, aliphatic and aromatic compounds and esters, organic and inorganic pigments and several types of resins whose basis is urethane, isocyanate, besides thinner, which contains benzene. Santos-Mello and Cavalcante (1992) performed a cytogenetic evaluation in gas station workers in the cities of São Paulo and Rio de Janeiro and found a significant increase in the frequency of chromosome deletions in the metaphases of the exposed individuals (0.829%), as compared to controls (0.126%).

Among mechanics, the custom of using petrol to clean their hands during car or engine repair is incessant; therefore, both mechanics and gas station workers inhale petrol gases which contain Genotoxic substances, such as benzene and the products derived from engine ignition. These products, when they enter the blood circulation, also present cytotoxic and genotoxic properties, as demonstrated by Hadnagy and Seemayer (1988).

Karahalil *et al*. (1998) evaluated workers of oil and petrol engine repair garages, using the Sister Chromatid Exchange (SCE) test and the presence of micronuclei in peripheral blood lymphocytes. The exposed group presented an average of 1.87 MN and the control group an average of 1.56 MN, which is a significant difference at the $p<0.05$ level. The results of both tests were in agreement with each other. They also agree with the data obtained in this study.

The smoking habit is widely accepted as a major cause of bladder cancer, since 50% of the cases were

identified in smoking men and 1/3 in smoking women. Burgaz *et al*. (1995) found a significant increase in micronucleated cells ($p<0.001$) in smokers, as compared to non-smokers. This is confirmed by the data obtained by us.

Alcoholic beverages have been described as containing mutagenic substances (Maluf and Erdtmann, 2000). Bishop *et al*. (1997) mention that alcohol does not induce mutations in mammal cells *in vitro*, whereas in vivo it induces a variety of genetic effects, including sister chromatid exchange and the production of micronuclei, where the evidence, however, is limited to certain test systems or tested organisms. According to Dittberner *et al*. (1997), alcohol use can increase the number of micronuclei. In the present work there is increase in the frequency of induction in the MN in the exposed individual consuming alcohol.

Stich and Rosin (1984) evaluated smoking and alcohol consumption over MN frequency, in order to detect the synergistic effect of these habits. They verified that smokers who drank alcohol presented a significantly increased frequency of MN in relation to the other interactions.

In cell cultures of peripheral lymphocytes, cytogenetic alterations like chromosomal aberrations, sister chromatid exchange and micronucleus induction have been applied as biomarkers of exposure and early effect in exposures to genotoxic carcinogens (Norppa, 2004; Albertini, 2000). The relevance of increased frequency of cytogenetic alterations as biomarkers of cancer risk has been corroborated by epidemiological studies suggesting the high frequency of chromosomal aberrations as the best predictive parameter for increased cancer risk (Franco *et al*., 2008).

Young workers in engine repair workshops are exposed to PAHs from engine exhaust and used engine oil. CA frequencies were found to be higher for exposed subjects ($p<0.05$). The levels of 1-OH-pyrene were higher not only in the exposed group compared with controls ($p<0.001$) but also in exposed nonsmokers compared with exposed smokers ($p<0.05$) (Karahalil *et al*., 1998). Smith *et al*. (1998) used painting probes for chromosomes 8 and 21. They observed an increase in the hyperdiploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 with exposure to benzene concentrations higher than 380 mg m^{-3} Zhang *et al*. (1998) used FISH in the same group to determine specific aberrations in chromosomes 1, 5 and7. Exposure to benzene was associated with increases in the rates of monosomy 5 and 7 and with increases in-the trisomy and tetrasomy frequencies of all three chromosomes. This result demonstrates that the leukemia specific changes in chromosomes 5 and 7 can be detected by FISH in the peripheral blood of healthy exposed workers.

Several organs are believed to be susceptible to tumor formation after exposure to PAHs. These include the lungs (in particular the bronchi), the skin, the esophagus and colon, the pancreas, the bladder and the breast in women.

In corroboration to these results our study supports the findings that the environ ment in motor garages is genotoxic to human and smoking habit increases the genotoxic effects of motor garage pollutants.

CONCLUSION

Genotoxic studies are foremost for any occupational exposure studies. Evaluation based on genotoxic parameters is often useful in warranting environmental endowment and occupational health.

The results of this study have shown increased chromosomal aberration as well as increased frequency of cells with micronuclei in motor garage workers as compared to non exposed controls. This indicates that occupational exposure to the many chemicals of the garage environment can have genotoxic effects and these effects increases with the increase in period of exposure.

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