Cytogenetic Biomonitoring among Petrol Filling Station Workers; A Hematological and Micronucleus Study

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ABSTRACT

Introduction: Workers of an occupational setting like gas stations are very prone to occupational health hazards with chemical substances present in the atmosphere of their workplace[1]. Screening and monitoring the gasoline related cytotoxicity in gas stations can be a useful tool in detecting initial cellular alterations. So this study was undertaken to detect early biological changes in specific target tissues like buccal mucosal cells and blood. Objective of the study was to explore the cytogenetic damage in exfoliated buccal cells and also damage at hematopoitic level with blood examination obtained from petrol station workers and control subjects, using micronucleus (MN) assay.

Material and Methods: This was a case-control study. The study population comprised of 90 male petrol pump workers (exposed group) and 30 unexposed controls (healthy individuals). Oral smear was obtained from normal mucosa by using the sterile cytopathological brush. Venous blood sample was collected for the assessment of Hb%, RBC, WBC, and TLC.

Results: More than one third (45.6%) of the cases were between 20-30 years of age. In 37.8% of the cases, the duration of work year was 5-10 years. The habit of smoking was among 24.4% of the cases while chewing was in 42.2% of the cases. The MN in Giemsa ($p=0.0001$) and in PAP ($p=0.001$) was significantly ($p=0.0001$) higher among the cases compared with controls. Hb was significantly ($p=0.001$) lower among the cases compared with controls. The level of other biochemical parameters like TLC and RBC count was similar among cases and controls. There was no significant ($p>0.05$) difference in the frequency of MN in Giemsa and PAP on the basis of work years and working hours/day of the cases.

Conclusion: In conclusion, our results reveal that petrol station workers could be under risk of significant cytogenetic damage.

Keywords: Micronucleus, Occupational exposure, Petrol pump workers, Genotoxicity

INTRODUCTION

Millions of workers in a variety of occupational settings are prone to be exposed to organic chemicals, intermediates, by-products or end products which possess health hazards. Petroleum derivatives are a complex combination of hydrocarbons and about 95% of compounds in petrol vapors are aromatic, aliphatic and alicylic compounds. It is supported by literatures that these products have genotoxic, mutagenic and carcinogenic potential. In 1988, the International Agency for Research on Cancer (IARC) considered all refinery environments potentially carcinogenic for human.[1] Epidemiological studies in workers of petrochemical industries and people living in the neighborhood exposed to gasoline vapors revealed a rise in pulmonary toxicity, neurotoxicity and cytotoxicity.[2-5] Since petrol is volatile in nature, it easily gets diffused in air and enters human body through inhalation at petrol filling stations. Although people are exposed to gasoline fumes during fuelling and refueling at gas stations, the gas station attendants are at higher risk of exposure by virtue of their occupation.[6] Also, the atmospheric concentration of gasoline vapor is not safe when inhaled even for a brief period of time and during fuelling of vehicles; the concentration of gasoline vapor in the air is recorded to reach between 20 and 200 ppm.[6,7] This concentration can get higher if there is a long queue of vehicles to be fuelled, which is a usual occurrence in countries like India also Petrol evaporates more readily in tropical than in temperate countries leading to rapid skin penetration and pulmonary absorption. In scientific literature there are several epidemiological studies worldwide which have shown correlation between benzene exposure in human with certain types of blood disorders.

In fuel stations, factories, refineries and other industrial settings benzene has been proven to be an important environmental contaminant. Most of the benzene is derived from the petrochemical industries. Exposure to benzene is seen at fuel stations. Moreover; the general population is exposed to benzene contained in petrol, vehicle exhaust, and diesel fuel. Occupational exposure to benzene has mainly been associated with increased incidences of blood disorders such as chronic myeloid and acute lymphoid leukemia and non-Hodgkin's lymphomas. Micronucleus (MN) assay for exfoliated cells in epithelial cells have been used to evaluate the genotoxic effects produced by doses of carcinogenic substances like benzene, to which human populations are exposed.[8,9] The frequency of MN in human exfoliated cells can be used as an "endogenous dosimeter" in tissues that are specific targets of genotoxic and carcinogenic agents, when carcinomas will develop.[10]

In Lucknow petrol pump workers are engaged in petrol filling for eight to twelve hours a day and do not wear protective equipment leading to higher opportunity for exposure. Personal hygiene and habits is also evident at work place, and therefore the occupational exposure to such derivatives like benzene adds up for genotoxic risk. This work aims to explore the cytogenetic damage in exfoliated buccal cells and also damage if any at the hematopoitic level with blood examination obtained from petrol

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station workers and control subjects, using micronucleus (MN) assay.

MATERIAL AND METHODS

Study design and Subjects

The study population composed of randomly selected 90 male petrol pump workers (exposed group) and 30 unexposed controls (healthy individuals). The exposed group included smokers and non-smokers, and chewers and non chewers and also workers either taking alcohol or not. The study group was from different petrol stations in Lucknow, Uttar Pradesh, India. The control (unexposed group) were all male, healthy individual without any systemic or mental illness. All subjects in the study group were given questionnaire which included questions about age, occupational exposure, smoking habit, chewing habit, use of drugs such as alcohol, virus illnesses any medication etc. All the individuals who agreed to participate in the study answered questionnaire. Study procedures used in the present study were approved by the Institutional ethical committee.

Method

Oral smear was obtained from normal mucosa by using the sterile cytopathological brush. The oral sites included buccal mucosa, hard palate, gingiva and floor of mouth. The smears were transferred and spread onto the labeled, clean, dry glass slide. Each slide was labeled with the patient's name. For Pap stain method, the slides were fixed at once by 95% ethanol for 20 minutes, whereas, they were air dried for Giemsa stain method. Two slides were made for each patient. One slide was stained by Papanicolau stain and the other by Giemsa stain. Observation was done in 10x, 40x and 100 x magnification, for the assessment of micronuclei. Venous blood sample was collected for the assessment of Hb%, RBC, WBC, and DLC. Venous blood by collected with the help of sterile needle and was collected in EDTA coated vials. Blood samples were stored in 4^0 Celsius temperature for assessment. Observation of stained blood films done in 10 x 40x and 100 x magnification for assessment of blood picture. Data recorded was subjected to statistical analysis.

STATISTICAL ANALYSIS

Results are presented in mean±SD and percentages. The non-parametric Mann-Whitney U test was used to compare two means and Kruskal-Wallis test was used to compare more two means. The Statistical analysis was done using SPSS 16.0 version (Chicago, Inc., USA). The p-value of less than 0.05 is considered as significant.

RESULTS

More than one third (45.6%) of the cases were between 20-30 years of age. In 37.8% of the cases, the duration of work year was 5-10 years and 31.1% worked for more than 10 years. The working hours was >10 hours/day among 56.7% and 6-10 hours/day among 32.2%. The habit of smoking was among 24.4% of the cases while chewing was in 42.2% of the cases. Less than 10% of the cases were smoker and chewer both. Only 11.1% were alcoholic (Table-1).

The MN in Giemsa was significantly (p=0.0001) higher among the cases compared with controls. Similarly, MN in PAP was also significantly (p=0.001) higher among the cases compared with controls (Table-2).

The Assessment of biochemical parameters among cases and controls are as follows Hb was significantly (p=0.001) lower among the cases compared with controls. The level of other biochemical parameters like TLC and RBC count was similar among cases and controls (Table-3).

There was no significant (p>0.05) difference in the frequency of MN in Giemsa and PAP on the basis of work years and working hours/day of the cases. MN in Giemsa and MN in PAP was significantly (p<0.01) higher among chewers compared with non-chewers. This was also significantly higher among individuals who did chewing along with smoking. Giemsa MN was significantly (p=0.02) higher among alcoholic than non-alcoholic (Table-4).

DISCUSSION

MICRONUCLEI are cytoplasmic chromatin masses appearing

	No. (n=90)	Percentage
Age in years		
20-30	41	45.6
31-40	33	36.7
>40	16	17.8
Mean±SD	32.53±8.96	
Duration of work in years		
1 year	9	10.0
1-5 years	19	21.1
5-10 years	34	37.8
>10 years	28	31.1
Working hours		
2-6 hours	10	11.1
6-10 hours	29	32.2
>10 hours	51	56.7
Smoking	22	24.4
Chewing	38	42.2
Both smoking and chewing	7	7.8
Alcoholic	10	11.1

Table-1: Basic characteristics of the cases

	Giemsa MN (Mean±SD)	PAP MN (Mean±SD)
Cases (n=90)	4.27±4.13	2.38±3.41
Controls (n=30)	1.30±1.53	0.47±0.86
p-value[1]	0.0001*	0.001*

[1]Mann-Whitney U test, *Significant

Table-2: Frequency of Giemsa MN and PAP MN in oral smear sample among cases and controls

	Cases (Mean±SD)	Controls (Mean±SD)	p-value[1]
Hb	13.78±1.38	15.02±1.17	0.001*
TLC	8373.11±1784.27	8471.67±784.65	0.77
P	67.99±6.41	66.27±5.30	0.18
L	29.74±6.65	30.60±6.43	0.54
E	1.56±1.04	2.13±0.86	0.007*
M	0.83±0.93	1.30±0.79	0.01
RBC	4.58±0.56	4.79±0.50	0.06

[1]Mann-Whitney U test, *Significant

Table-3: Assessment of biochemical parameters among cases and controls

	Giemsa MN (Mean±SD)	PAP MN (Mean±SD)
Duration of work in years		
1 year	2.33±1.65	1.00±1.32
1-5 years	3.53±3.89	1.79±2.87
5-10 years	3.65±2.81	1.74±2.06
>10 years	6.14±5.48	4.00±4.79
p-value[1]	0.10	0.11
Working hours		
2-6 hours	5.00±4.08	2.80±3.25
6-10 hours	4.79±5.25	2.79±4.64
>10 hours	3.82±3.39	2.06±2.54
p-value[1]	0.58	0.59
Smoking		
Yes	4.95±4.46	2.68±3.38
No	4.04±4.03	2.28±3.43
p-value[1]	0.32	0.54
Chewing		
Yes	6.11±5.08	3.74±4.45
No	2.92±2.59	1.38±1.88
p-value[1]	0.001*	0.009*
Both smoking and chewing		
Yes	9.00±5.22	5.14±1.97
No	3.87±3.80	2.14±3.26
p-value[1]	0.005*	0.01*
Alcohol		
Yes	7.22±5.16	3.56±4.18
No	3.94±3.90	2.25±3.31
p-value[1]	0.02*	0.22

[1]Mann-Whitney U test, *Significant

Table-4: Frequency of Giemsa MN and PAP MN in oral smear sample among cases by duration and time of work and addiction habit

as small nuclei that arise from chromosome fragments or intact whole chromosomes which lag behind in anaphase stage of cell division. Presence of Micronuclei in cytoplasm of cell depicts structural or numerical chromosomal aberrations.[11]

Our study showed significantly higher genotoxic or chromosomal damage in the form of emergence of MN in gasoline workers, supported by Evans 1997.[12,13] Volatile benzene and ethanol compounds in petroleum has been proven to be a potent carcinogen.[14]

Our study showed higher frequency of MN count in Giemsa than in PAP due to there is better resolution of nucleus in Giemsa stain. Giemsa stain is used in Giemsa banding, commonly called G-banding, to stain chromosomes. It can identify chromosomal aberrations as it attaches itself to regions of DNA.[15] Benzene produces hematological changes ranging from pancytopenia to total bone marrow aplasia, effected through its myelotoxic action (d'Azevedo et al, 1996)[16] That's why hematological analysis was included in our study. Our study revealed decreased Hb% and DLC in study cases as compared to controls.

The decreased DLC count in our study maybe a result of impaired migration of phagocytic cells, lower resistance to viruses, bacteria and irritant foreign bodies as supported by another study.[17] The decreased Hb% in study group is due to toxic components of petroleum fumes which have been reported to change blood chemistry and induce anemia by causing bone marrow depression similarly as was seen in studies by Marieb,1995; Rabble et al, 1996; Synder and Hedli, 1996.[18]

On the basis of work years and working hours/day, there was not much difference in the frequency of Micronucleus as seen in Giemsa and PAP in our cases this may be due to individual's own genetic susceptibility to benzene toxicity. Every individual has different potency of DNA repair mechanism which controls gene polymorphism, DNA stability and repair as suggested by Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzene (1995).

Increase in MN frequency is raised in smokers as compared to non smokers in our study and was similar with findings of Naushin. J. N et al.[19]

The number of MN count was raised in tobacco chewers than smokers. Tobacco with gasoline exposure causes increase in rate of frequency of MN similar to study.[20]

In our study chewers group was highest as compared to smokers and alcoholics and according to (Ramaesh T et al 1998)[21] chewing form of tobacco releases more nicotine than smoked form,which is directly related to the increased tobacco addiction.

CONCLUSION

In conclusion, our results reveal that petrol station workers are under continuous exposure to benzene related toxicity at cellular level.

The presence of micronuclei in exfoliated buccal epithelial cells has proven a useful biomarker of occupational exposure to petroleum products. Workers may be at higher risk of developing cancer at later stage, therefore should be carefully monitored for a long term effect of exposure.

In our study HB% and DLC count was found to be low, which can be taken into account for its relation with benzene exposure and so further studies can be carried out in this regard.

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