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Chromosomal Damage Risk Assessment to Benzene Exposure among Gasoline Station Workers in Bangkok Metropolitan, Thailand

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Abstract

This study was a cross-sectional survey study to assess relative risk (RR) of chromosomal damage through benzene exposure in 45 gasoline stations workers compared to 30 controls in central area of Bangkok. Sister chromatid exchange (SCE) is as genotoxic biomarker, performed in white blood cells, and blood benzene level (BBL) is a biological marker of benzene exposure was performed by gas chromatography-flame ionization detector (GC-FID) using modified headspace solid-phase micro-extraction (HS-SPME) technique. The results showed that the average blood benzene level of these workers was significantly higher than in the controls ($p < 0.001$) as well as the frequency of sister chromatid exchange. The sister chromatid exchange was strongly and positively associated with blood benzene level of gasoline workers ($p < 0.001$) with the chromosomal damage relative risk at 2.50 ($p < 0.001$).

Keywords: gasoline worker, benzene, sister chromatid exchange, chromosomal damage

1. Introduction

Air pollution has become to be a serious health problem in Bangkok, Thailand, especially among occupational workers such as gasoline workers. Exposure to gasoline vapors is classified by the International Agency for Research on Cancer as possible cancer risk in humans, mainly on the basis of the established carcinogenicity of some chemical components such as benzene (IARC 1989). The mechanism of benzene toxicity, particularly its leukemogenic effects, is far from being fully understood. Contamination of the environment with volatile organic compounds (VOCs) has become an important issue, since many of these compounds are toxic and may pose health risks of various concerns**.** To assess the biological risks caused by gasoline vapor, by biological monitoring using biological marker of oxidative chromosomal damage and repairing capacity (Lambert *et al.* 1982; Carrano *et al.* 1983), as frequency of sister chromatid exchange (SCE) in gasoline workers, may provide useful information about the genotoxic risk associated with exposure to this carcinogenic agent that is benzene. In major cities in Asia, the levels of ambient air benzene are relatively high compared with those in Europe or in the United States (Leong & Laortanakul 2003) and there is high prevalence of cancer and leukemia related to petrol station density (Yimrungruang *et al.* 2008*;* Chang *et al.* 2009; Weng *et al.* 2009)**.** The purpose of this study was to evaluate the relative risk

(RR) of chromosomal damage cause by benzene exposure in gasoline station workers in central area of Bangkok, Pathumwan district, comparing to controls.

2. Research Methods

2.1 Population study

A cross sectional surveyed at 11 gasoline stations in Pathumwan district, Bangkok, Thailand, and collected blood samples analysis from 45 gasoline workers, which 33 non-smokers and 12 smokers, compared to 30 non-smoker controls from April to June 2009. All subjects have given informed consent before the study. The Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, approved the study. All subjects were healthy and had worked more than six months.

2.2 Sample collection

The venous blood samples were drawn from subjects during the 6-8 h shift work, 2 x 2 mL, using plastic heparinized vacuum blood tube, stored at 4 °C, one SCE frequency analysis within 6 h and the other one stored at -20 °C before benzene analysis within 1 month.

2.3 Sample analyses

The blood examination for SCE frequency was modified in manner suggested by Tucker and Preston (1996). Aliquots of 0.5 mL of heparin zed blood samples, both worker and control groups, added to 5.0 mL of the culture medium containing Roswell Park Memorial Institute medium (RPMI 1640, Hyclone, Utah, USA), supplemented with 15% fetal bovine serum, 2.5% phytohemagglutinine (PHA, Sigma, Germany) and 1% penicillin-streptomycin. Afterwards 100 µL of 1.3 mg/L 5′-bromodeoxyuridine (BrdU, Sigma Chemical Co.) was added to the medium and additionally incubated in the dark room temperature for 96 hours. Immediately, added colchicine (0.2 µg/L, Sigma Chemical Co.), collected cultured cells and treated with 0.075 mol/L potassium chloride (KCl) at 37 °C for 10 min to be fixed with methanol-acetic acid (3:1). Standard harvest procedure was performed by a drop of harvested cell pellets spread on clean glass slide and stained by Hoechst No.22358 plus Giemsa technique (Koto *et al.* 1975). Finally, the slides were examined by a light microscope (Nikon E200) in regard to SCE frequency/metaphase cell. The total of 15 well-spread metaphases was evaluated in worker and control groups. Counting of SCE frequency was done by using oil immersion.

Blood benzene determination was performed by GC-FID using modified HS-SPME technique (Tunsaringkarn *et al.* 2004). Briefly, to 0.5 mL of blood sample in glass cap bottle was added 0.2 g of sodium chloride, shaking vortex for 15 s, then controlled and absorbed by SPME in water bath at 50 °C with vibrator for 20 min. Injected in GC (Varian CP 3800) at 220 °C with flame ionization detector at 220 °C (used column CP-SIL5 CB, split less). Oven temperature started at 50 °C for 10 min, then increased 5 °C/min until 90 °C and finally increased by 30 °C/min until 250 °C for 17 min. The quantity of blood benzene was analyzed under relative intensity of chromatographic signal for 40 min. The Limit of Detection (LOD) of benzene was 10.00 µg/L (ppb) and the average coefficient of determination (r2) was 0.999657.

Statistical analyses were carried out with the SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used for BBL and frequency of SCE in gasoline worker and control groups which were presented as mean and standard error of the estimate (Mean ± SE). The comparison between control and worker parameters were analyzed by independent-t test with a value $p < 0.05$ limitation. The association between parameters and SCE was the estimated relationship of them by multiple linear regression, which SCE as dependent variable, BBL, age, sex and cigarette smoking as independent variables. The relative risk (RR) of chromosomal damage was calculated by comparing the proportion of

workers with frequency of SCE higher than the mean plus standard error value of control to proportion of workers with frequency of SCE lower than the mean plus standard error value of the control group. Chi-square analysis was used to determined differences between groups in frequency distribution with 95% confidence interval (CI).

3. Analysis results

A total of 75 subjects, 30 controls and 45 gasoline station workers, were included in this study. The average age of worker and control groups was 31.2 ± 1.4 and 31.2 ± 1.8 years. The gasoline workers were 60% male and 26.7% smoker while the controls were 60% male and all of them were non-smokers. The frequency of SCE in workers and controls was 13.62 ± 0.24 and 6.97 ± 0.20 SCE/cell (Table 1 and Figure 1). Both, smoking and non-smoking workers, SCE frequencies were significantly higher than those of controls ($p < 0.001$). SCE frequency in smokers had higher level than in non-smokers but there was no significant difference. The average BBL of workers and controls was 302.84 ± 20.54 and 27.28 ± 15.31 µg/L, but workers had significantly higher levels than controls ($p < 0.001$) as shown in Table 1. But SCE and BBL of male and female were not difference in each group. In addition, the benzene exposure risk ratio (BBL of workers/controls) was 11.83 while the chromosomal damage risk ratio (SCE of workers/controls) was 1.94.

The association between BBL and SCE frequency was analyzed by multiple linear regression with SCE as dependent variable and BBL, age, sex and cigarette smoking as independent variables (Table 2). The frequency of SCE was positively and significantly associated with BBL ($p = 0.001$). Age, sex and cigarette smoking, were not significantly associated with SCE ($p > 0.05$). The frequencies of SCE were dichromatomized into high- and low-frequency groups based on their means plus standard error of estimate (SE) values of control group at 7.17 (6.97 + 0.2). The chromosomal damage relative risk of gasoline station workers compared to controls was 2.50 (χ2–test, 95% CI = 1.17-5.34, $p < 0.001$).

4. Discussion

Volatile organic compound in gasoline is a common source of benzene which is classified as carcinogen. It can cause serious health effects including genotoxicity. Biomarkers of response indicate biological or biochemical changes in target tissue or surrogate from chemical action. The biomarkers commonly used are chromosome aberration (CA), micronucleus (MN) and SCE. This study used SCE as genotoxic biomarker and BBL as exposure biomarker. The results of this study showed significantly higher genotoxic or chromosomal damage in gasoline workers than in controls which supported the previous studies (Vijayalaxmi & Evans 1982; Celik & Akba 2005; Calderón-Ezquerro *et al.* 2007). The SCE frequency was not effective for low smoker workers with less than 10 cigarettes a day (average number of cigarette smoking 5.2 cigarette/day), but there was a trend of higher chromosomal damage in smokers than in non-smokers. The comparison between smokers and non-smokers should be related to the number of cigarettes smoked a day with a significant difference in moderate and heavy smoker (Vijayalaxmi & Evans 1982; Celik & Akba 2005; Calderón-Ezquerro *et al.* 2007). The BBL of all workers was significantly higher than in controls ($p < 0.001$), but BBL of smokers trending upwards compared to non-smokers, but not significantly different, most non-smokers being women with higher heart rate than men (Ryan *et al.* 1994; Stein *et al.* 1997). As the results of women were higher in BBL than in men with same rate of benzene clearance, it may influence its internal exposure and cancer development. Gender affected more BBL than benzene exposure from low cigarette smoking. In addition, BBL of all gasoline workers were higher than the biological monitoring of occupationally exposed persons, an exposure equivalent for carcinogenic working material (EKA-value) of 54 µg/L (Angerer *et al.* 1991). It should be considered that BBL in workers was 5.6 folds of limited level, corresponding to high level of benzene exposure (11.83 folds of exposure risk ratio) and this marker indicating that cells have been exposed to mutagen or carcinogen (Keretetse *et al.* 2008). The frequency of SCE provided information of cumulative effects of carcinogens which was associated with increased risk of cancer (1.9 folds of chromosomal damage risk ratio). The results of this study indicated that the frequency of SCE was strongly associated to BBL ($p < 0.001$). With

every increase of one year in age, the average SCE decreased by 0.25 whilst every increasing of BBL level 1 μg/L, the average SCE increased by 0.44. In fact, the average age of control group was not higher than the average in the worker group. Age, cigarette smoking and sex were not significantly associated to chromosomal damage. Some studies showed that no correlation between SCE frequency, duration of exposure, smoking habit, and age (Hoet *et al.* 2009; Ulker *et al.* 2008). The gasoline workers had an increased relative risk of chromosomal damage at 2.50 compared to controls (χ2–test, 95% CI = 1.17-5.34, $p < 0.001$), which benzene exposure and chromosomal damage risk ratios of the workers were 11.93 and 1.94, respectively.

However, biomonitoring in peripheral lymphocytes served as an early indicator of chromosomal damage. Biomonitoring of benzene exposure by using BBL among the gasoline workers should show the association of cancer risk development in gasoline workers prompting to plan a screening program and primary prevention for them to minimize the risk of cancer. Future studies of genotoxicity in smokers and non-smokers comparison should cover a large population and take into consideration confounders such as age, gender, smoking habits, alcohol consumption and family history of cancer.

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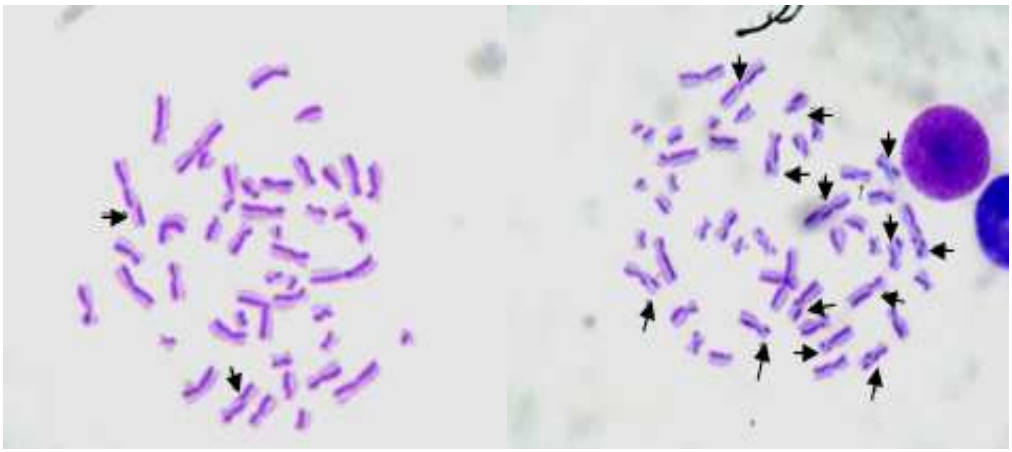
*Statistical difference (independent-t test) between control and gasoline workers					
Parameter	**Control**		**Gasoline Worker**		***p* - value***
	N	**(Mean ± SE)**	**N**	**(Mean ± SE)**	
Age (years)	30	31.2 ± 1.8	45	31.2 ± 1.4	< 0.001
Frequency of SCE (SCE/cell)	30	6.97 ± 0.20	45	13.62 ± 0.24	< 0.001
Sex					
Female	12	7.28 ± 0.13	18	13.41 ± 0.33	< 0.001
Male	18	6.77 ± 0.31	27	13.76 ± 0.34	< 0.001
Cigarette Smoking					
Non Smoker	30	6.97 ± 0.20	33	13.47 ± 0.26	< 0.001
Smoker		-	12	14.07 ± 0.59	
Blood Benzene (µg/L)	30	27.28 ± 15.31		302.84 ± 20.54	0.001
Sex					
Female	12	36.65 ± 36.65	18	322.26 ± 0.26	0.001
Male	18	21.03 ± 12.10	27	291.05 ± 20.91	0.001
Cigarette Smoking					
Non Smoker	30	27.28 ± 15.31	33	201.97 ± 24.78	0.001
Smoker	-	-	12	321.33 ± 25.96	
Benzene Exposure Risk Ratio (BBL of workers/controls) = 11.83					
Chromosomal Damage Risk Ratio (SCE of workers/controls) = 1.94					

Table 1. BBL and frequency of SCE in controls and gasoline workers

Dependent Variable: SCE *Statistical relation between SCE and parameters					
Parameter	**Unstandardized**		**Standardized Coefficients**	**95% CI Lower to Upper**	***p* - value***
	B	**SE**			
Blood Benzene Level	0.008	0.002	0.439	0.004 to 0.012	0.001
Age	-0.064	0.033	-0.249	-0.131 to -0.004	0.063
Sex	-0.101	0.700	-0.016	-0.1506 to 1.305	0.886
Cigarette Smoking	1.460	0.879	0.202	-0.222 to 3.142	0.087

Table 2. Association between SCE frequency and parameters



(a) Control group (b) Gasoline worker group

The symbol (→) indicated sister chromatid separation.

Figure 1. Sister chromatid exchanges of control and gasoline worker groups