BLOOD TOLUENE AND GENOTOXICITY IN GASOLINE STATION WORKERS IN BANGKOK: A PRELIMINARY STUDY

Tanasorn Tunsaringkarn[1, *], Jamsai Suwansaksri[2], Panthira Ketkaew[3], Wattasit Siriwong[1]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2]Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[3]Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140 Thailand

ABSTRACT: Toluene is widely used as an industrial solvent and is usually produced in the processes of making gasoline. Inhaled toluene may cause genotoxicity and other adverse health effects. This study aimed to ascertain the relation between blood toluene exposure and genotoxicity, as measured by sister chromatid exchange (SCE), by comparing gasoline station workers to controls. Mean blood toluene and SCEs in gas station workers were 225.06 mg/L and 13.62 SCEs/cell, respectively which they were significantly higher than those in controls ($p=0.001$ and $p<0.001$, respectively). Also, linear regression analysis showed a significant positive relation ($p<0.01$) between blood toluene and SCEs. Age was inversely related to SCEs ($p<0.01$). In conclusion, SCEs may be supported the warning of genotoxicity by toluene exposure in gasoline station workers.

Keywords: Genotoxicity, SCE, Toluene

INTRODUCTION

Toluene is highly lipophilic, which accounts for its primary effects on the central nervous system (CNS). CNS symptoms depend on duration, route of exposure, and level of toluene in the air or solvent. Prolonged exposure to toluene by inhalation is associated with heart, liver, kidney, and lung damage [1, 2] but toluene is still only a questionable carcinogen [3]. Most human carcinogens are genotoxic but not all genotoxic agents have been shown to be carcinogenic in humans. Genetic monitoring of human populations exposed to potential mutagens/carcinogens can provide an early warning system for genetic disease or cancer. Induced chromosomal changes in human lymphocytes as well as SCEs are well-established biomarkers of occupational or environmental exposure to genotoxic agents [4, 5]. SCE is the exchange of genetic material between two identical sister chromatids, and is used in mutagenic testing of many products. SCE occurs normally in cells during cell division, but when a cell's DNA is damaged by genotoxic agents, the rate of SCE increases. It is thought that SCE is an attempt by the cell to repair the DNA damage caused by genotoxic agents [6, 7]. Frequent SCEs also be related to formation of tumors or cancer development. Gasoline station workers are exposed to several genotoxic compounds in gasoline vapor. These compounds, including toluene, are of public health concern. The relation between SCEs and blood toluene could prove useful to assess genotoxicity in occupational toluene exposure. This study aimed to evaluate the relation between SCEs and blood toluene in gasoline station workers in Bangkok.

** Correspondence to: Tanasorn Tunsaringkarn*
E-mail: tkalayan@chula.ac.th

MATERIALS AND METHODS

Population Study

A cross sectional survey collected EDTA blood samples of 46 gasoline station workers of all 11 gasoline stations in Pathumwan district compared to 10 controls of government official workers during April to June 2009 which they worked in the same area, central Bangkok, Thailand. All subjects were more than 18 years old, and had worked in their stations or offices more than 6 months. All subjects gave informed consent before the study. The study was approved by the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University. All subjects were healthy and willing to participate in this study.

Blood Collection

Four ml of venous blood of workers were collected in heparinize glass vacuum tubes at 8 hours after the start of their work-shifts [8], and stored at -20^{o}C before toluene analysis. SCEs were measured by incubating blood samples in cell culture media of RPMI 1640 with glutamine complete fusion medium within 6 hours after blood collection [9].

Laboratory Analyses

SCE analysis

All samples were analysed using the method described by Tucker and Preston [10] plus Giemsa

Table 1 Bivariate comparison of subject characteristics in gasoline station workers and controls

Parameters	Controls		Gasoline workers		*P-Value**
	n	mean ± SE or %	n	mean ± SE or %	
Frequency of SCE (SCEs/cell)	10	6.97 ± 0.20	46	13.62 ± 0.24	<0.001
Men	6	6.77 ± 0.31	30	13.71 ± 0.33	<0.001
Women	4	7.30 ± 0.10	16	13.46 ± 0.35	<0.001
Blood Toluene (μg/L)	10	47.71 ± 17.30	46	225.06 ± 23.05	0.001
Men	6	79.51 ± 19.93	30	185.12 ± 22.71	<0.01
Women	4	0.00 ± 0.00	16	304.93 ± 46.79	<0.001
Age (years)	10	51.2 ± 1.8	46	31.2 ± 1.4	<0.001
Cigarette Smoking n (%)	10	0 (100.0)	46	12 (26.1)	0.071
Alcohol Drinking n (%)	10	5 (50.0)	46	22 (47.8)	0.950

* Significant difference between control and worker groups

Table 2. Linear regression analysis of SCE frequency and blood toluene levels in gasoline station workers

Parameter	Unstandardized Coefficients		95% CI	*P-Value*
	B	S.E.	Lower to Upper	
Toluene	0.007	0.002	0.020 to 0.012	0.004
Gender	-0.714	0.738	-2.196 to 0.768	0.338
Age	-0.094	0.032	-0.159 to -0.030	0.005
Cigarette Smoking	1.221	0.885	-0.557 to 3.000	0.174
Alcohol Drinking	0.988	0.695	-0.408 to 2.384	0.202

Dependent Variable: SCE

technique [11]. Briefly, 0.5 ml of heparinized blood sample was incubated with 5-bromodeoxyuridine for 96 hours at room temperature. Colchicine (final concentration, 0.2 μg/ml) was immediately added before harvesting lymphocytes for arresting cells in metaphase and stained by Hoechst No.22358. For each case, 15 well-spread metaphases were scored for SCE. Counting was done using oil immersion. All analysis was performed at the Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Blood toluene analysis

The analysis was performed using headspace-solid phase microextraction (HS-SPME) technique and GC-FID by Tunsaringkarn et al. [12] method. The LODs (Limit of Detection) of toluene as 5.00 μg/L (ppb) and coefficient of determination (r^2) was 0.999270. All analysis was performed at the Department of Chemistry, Faculty of Science, King Mongkut's University of Technology, Thonburi, Bangkok, Thailand.

Statistical Analysis

Bivariate and multivariable analyses were conducted. In bivariate analysis, independent-samples t-tests were used to compare mean SCE frequency, toluene level, and age between the gas station workers and the control subjects. Chi-square tests were used to compare these groups with respect to smoking and alcohol drinking. A multivariable linear regression model was also constructed, with SCE as the dependent variable and toluene level, age, smoking, and drinking as independent variables. Analysis was conducted using SPSS for Windows, version 17.0. The critical level (alpha) was 0.05.

RESULTS

Table 1 shows results of the bivariate analysis. Mean SCE frequency and toluene level were significantly higher in gasoline station workers than controls (*p<0.001*). Mean age was significantly lower in gasoline station workers. Mean toluene level was 4.7 times higher in gasoline station workers. Table 2 shows results of the linear regression analysis. Toluene level was significantly positively associated with SCE frequency; each μg/L increase in toluene level was associated with an increase of 0.007 SCEs/cell. Age was significantly negatively associated with SCE frequency. Smoking, alcohol drinking and gender were not significantly related to SCE frequency.

DISSCUSSION

Toluene (methylbenzene) is a common volatile in many industrial processes [13] for which ATSDR [14] have documented and recommended the monitoring for high risk groups. Previous studies found differences in aberrant cells and chromatid breaks between workers exposed to toluene at working air concentrations in the range of 104 - 1170 ppm (390 - 4380 mg/m^3) in a rotogravure printing plant and controls, [15] and observed a strong relationship between the individual toluene burden and the genotoxic risk of the exposed persons [16]. Toluene genotoxicity in printing workers study showed significant higher aberrant cell of peripheral lymphocyte in workers than control group *(p<0.05)* [17] but the cytogenetic response (SCEs) did not differ between policemen

with and without high toluene exposure [18]. Gasoline station workers are a relatively high-risk group for toluene exposure; a recent study showed higher ambient toluene exposure (490.38 - 944.77 μg/m^3) than benzene (55.22 - 292.52 μg/m^3), m,p-xylene (40.79 - 154.54 μg/m^3), ethylbenzene (22.64 - 52.42 μg/m^3) and o-xylene (15.54 - 49.90 μg/m^3) [19]. Several studies have suggested that solvents might be cause of increasing of chromosomal damage in gas station attendants [7, 20], but there was no report on the association between toluene exposure and chromosomal change in white blood cell of gasoline workers.

This study presented blood toluene exposure and SCEs of gasoline workers were significant higher than controls ($p \leq 0.001$) which blood toluene in all workers were higher 4.5 folds of toluene Biological Exposure Indices (BEI) reference values (50 μg/L) [21]. This result supported high risk of toluene exposure in gasoline workers. In this study, age was also significantly (negatively) associated with toluene level. Thus inclusion of age in the linear regression model adds confidence to the observed positive association of toluene level with SCE frequency. With every increase of one year in age, the average SCEs decreased by 0.094.

This study had several limitations. There are several volatile organic compounds (VOCs) in ambient air such as BTEX (benzene, toluene, ethylbenzene and xylene) which the gasoline station workers exposed during their work shifts. The previous in vitro experiments found that neither benzene, toluene nor xylene changed the number of sister-chromatid exchanges (SCEs) or the number of chromosomal aberrations in human lymphocytes. Toluene and xylene caused a significant cell growth inhibition which was not observed with benzene in the same concentrations [22]. So, it's difficult to absolutely conclude that toluene directly effected to chromosomal damage. Moreover, the number of control subjects in this study was low and their mean age was significantly higher than that of the gas station attendants. Hagmar et al. [23] proposed that age, sex and duration time of exposure did not affect chromosomal damage but studies by Bolognesi et al. [24] and Bukvic et al. [25] showed age positively related to increase chromosomal damage. However, this study showed that age inversely related with SCE frequency. Based on this information, age was important confounding factor, but gender did not show relation to SCEs. The other genotoxicity tests such as chromosome aberrations (CAs), fluorescence in situ hybridization (FISH) and micronucleus formation (MN) were recommened [26] in the large number of subjects (gasoline station workers and controls) in future genotoxic effect of toluene exposure study. The confounding factors such as age, gender, smoking and alcohol drinking should be considered and might explain some individual variation of effects. Also, in this study both toluene level and age exhibited vary large differences between the two study groups. These differences lend uncertainty to the interpretation of the regression analysis. For example, the reported regression relationship between toluene level and SCE frequency could well be an overestimate.

In conclusion, our results showed blood toluene positively related to increase of the chromosome damage in lymphocytes and emphasize the need to take into account the potential confounding effect of this variable in the design of biomonitoring studies based on chromosome damage. SCE was usefulness of cytogenetic biomarkers as intermediate end points in carcinogenesis which can be caused by genetic mutation. In fact, a series of several mutations in certain classes of genes is usually required before a normal cell will transform into a cancer cell [27]. Further research should be conducted to evaluate associations of toluene and other BTEX compounds with genetic changes. If the present results are confirmed, biomonitoring of toluene exposure and effect are recommended in workers with substantial toluene exposure.

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