Original article

Chromosome aberrations and DNA damage in petrol pump workers in Chiang Mai

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Objective The objective of this study was to investigate the genetic damage in petrol pump workers, who were exposed occupationally to gasoline, its derivatives, and air pollution through inhalation and skin contamination, and to compare them with control subjects.

Methods The chromosome aberration test and single cell gel electrophoresis (comet assay) were used to evaluate the DNA status in peripheral blood lymphocytes of 32 petrol pump workers from eight different gasoline stations in central Chiang Mai, Thailand. Thirty control subjects, who had not been exposed occupationally to benzene, were matched to the exposed subjects by gender and age.

Results The comet assay revealed that DNA damage in the peripheral blood lymphocytes of petrol pump workers was signifi cantly higher than that in the controls. The average tail length and tail moment in the exposed workers were 5.51±5.46 μm and 1.85±2.67 μm, respectively, based on 100 cells/individual, whereas, the average tail length and tail moment in the control group were 1.57±1.03 μm and 0.31±0.27 μm, respectively. In addition, results of the chromosome aberration test showed that the frequency of cell with aberrations in exposed workers was significantly higher than that of control subjects, 1.87% vs. 1.06%, respectively.

Conclusion This study found a significant excess of DNA damage and structural chromosome aberrations in workers who were exposed occupationally to gasoline vapor and air pollutants, when compared to the matched controls. These fi ndings confirm the potent genotoxic effect of gasoline and air pollutants as an inducer of DNA damage and structural chromosome aberrations.
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Keywords: DNA damage, chromosome aberration, comet assay, petrol pump worker

Introduction

Several epidemiological and environmental studies have been conducted on human populations exposed to genotoxic agents in their workplaces. These studies have shown that there is an increased frequency of DNA and chromosomal damage. Anwar evaluated chromosome aberrations in traffic policemen in Cairo, Egypt, and found that the percentage of chromosome aberrations was significantly higher among traffic policemen than in the control group [1]. Furthermore, the incidence of lung cancer in Cairo has increased, which is related to air

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pollution. A corresponding study in Dhaka city, Bangladesh, was performed to determine the level of PAH-DNA adducts in white blood cells, a marker of exposure to environmental and occupational polycyclic aromatic hydrocarbon, in 46 three-wheel drivers and 48 non-drivers [2]. The study showed that drivers had a significantly higher white blood cell PAH-DNA adduct level than the non-drivers. The results suggest that urban residents, who are exposed occupationally to traffic pollution, are at a potentially higher risk of health effects than those who are not exposed to it.

Petrol pump workers are exposed occupationally to genotoxic agents because of gasoline, which includes a large number of organic chemicals such as benzene, toluene, and other monocyclic, aromatic, aliphatic and polycyclic aromatic hydrocarbons [3,4]. Several studies have shown that benzene can induce various forms of genetic damage including chromosome aberrations, sister chromatid exchanges (SCE), micronuclei formation and DNA damage. Chung and Kim suggested that the benzene metabolites, 1,2,4-benzenetriol, hydroquinone, and trans, trans-muconic acid induced chromosome specific numerical and structural aberrations [5]. Consequently, benzene is classified as a human carcinogen and mutagen. It is widely found in the workplace and environment, and is an established cause of hematotoxicity, leukemia and other bone marrow disorders in humans [6-8]. Single cell gel electrophoresis is a rapid test in biomonitoring occupational exposure to DNA damaging agents [9,10]. Andreoli et al performed the comet assay on peripheral blood lymphocytes of gasoline station attendants who were exposed to benzene [11]. The result revealed a significant excess of DNA damage the in lymphocytes of exposed workers compared to matched unexposed controls. They also concluded that the comet assay is a sensitive technique for detecting DNA damage at the single cell level.

In 2007, Chiang Mai, Thailand, had a population of about 1,664,399 people [12]. Additionally, its land transport data showed an increase in motor vehicles from about 750,772 to 939,759 between 2006 and 2010 [13]. Therefore, the demand for gasoline delivery increased. Occupational exposure to mutagenic and carcinogenic agents creates a significant impact on the health status of gasoline station attendants. Biomonitoring studies on these workers are essential. The DNA status in their lymphocytes may be a predictor of future cancer risks and might help to prevent further deterioration in the health of these workers.

Methods

Thirty two male workers (18-25 years old), from eight petrol pumps in central Chiang Mai, Thailand, having been exposed to the ambient air, were matched with and 30 male control individuals in respect to age and sex. The controls had no known chemical exposure at work (Table 1). Only those participants who had not received drug treatment or x-rays during three months before sampling were selected.

Informed consent was obtained from all subjects according to the ethics committee of the Faculty of Medicine, Chiang Mai University.

Chromosome aberration test

The chromosome aberration test was performed according to the guidelines of Carrano and Natarajan, [14] with a slight modification. Blood samples were obtained by venipuncture using heparinized syringes. Lymphocyte cultures were established by adding 0.25 mL of whole blood to five mL of culture media, supplemented with fetal bovine serum, phytohemagglutinin and antibiotics. The cultures were incubated for 48 hours at 37 °C. Preparation of chromosomes was carried out according to the conventional method. The metaphase chromosomes were stained with a conventional Giemsa staining technique. A total of 100 randomly selected cells at metaphase were analyzed from each subject.

Single cell gel electrophoresis

The comet assay was performed under alkaline conditions, following the procedure of Singh et al [15] with a slight modification. From each subject, a freshly prepared suspension of lymphocytes in 0.75% low melting point agarose was added to the frosted part of a microscopic slide, precoated with 1% normal melting point agarose. The cells were lysed in an alkaline buffer for 1 hour at a pH of 10. The slides were placed in an electrophoresis alkaline buffer (pH 13) for 20 minutes, thus allowing the DNA to unwind. Electrophoresis was performed for 20 minutes using a power supply of 18 V and 350 mA. After electrophoresis, the slides were washed in a neutralized buffer (pH 7.5) three times, each for 5 minutes, and stained with

Table 1. General characteristics of the petrol pump workers and control subject

Parameters	Control subjects	Exposed workers
Number of subjects	30	32
Mean age (years)	20.20 ± 0.77	21.10 ± 1.74
Gender	male	male
Duration of work		
3 months – 1 year	-	24
1 year – 5 years	-	8
Alcohol consumption		
Yes	2	4
No	25	16
Abstinent	3	12
Cigarette smokers	1	10
Non-smokers	29	22

ethidium bromide (0.1 mg/mL). The slides were examined under a fluorescent microscope (Axioskop 2, Carl Zeiss) equipped with an excitation filter of 515-560 nm, and a barrier filter of 590 nm, using 400X magnification. The computerized image analysis used the "Comet Imager" program (MetaSystems). A total of 100 randomly selected comet cells for each sample was captured. The comet parame-ters; tail length, tail intensity and tail moment were chosen to represent the dataon genotoxic effects.

Statistical analysis

The chi-square test was used to detect the difference between chromosome aberrations in the exposed and control groups, and the Mann-Whitney U test was used for comparing the DNA damage evaluated by comet assay.

Results

Results of the comet assay showed that DNA damage in the peripheral blood lymphocytes of exposed workers was significantly higher than that in the controls (p<0.001). In the exposed group, the tail length and tail moment varied between 1.16 and 23.9 µm, and 0.13 to 10.92 µm, respectively. On the other hand, the tail length and tail moment in the control group ranged from 0.3 to 4.28 µm, and 0.01 to 1.08 µm, respectively. The average tail length and tail moment in the exposed group, based on 100 cells/individual, was 5.51±5.46 µm and 1.85±2.67 µm, respectively. Whereas, the average tail length and tail moment in the control group was 1.57±1.03 µm and 0.31±0.27 µm, respectively (Table 2). In addition, results of the chromosome aberration test showed that the frequency of cells with aberration in the exposed workers was significantly higher than that of control subjects (p<0.012). The chromosome type aberration was higher in petrol pump workers than in the control, but it was not significantly different (p<0.87). However, the chromatid type aberration in petrol pump workers was significantly higher than that in the control subjects (p<0.004) (Table 3). There was no significant difference in DNA damage between non-smokers and smokers in the petrol pump worker

Table 2. Ranges and means of tail length and tail moment in the control and exposed group

Parameters	Group		*p* value
	Control (N=30)	Exposed (N=32)	
Tail length			
Range (µm)	0.30 - 4.28	1.16 - 23.90	
Mean ± SD (µm)	1.57 ± 1.03	5.51 ± 5.46	< 0.001
Tail moment			
Range (µm)	0.01 - 1.08	0.13 - 10.92	
Mean ± SD (µm)	0.31 ± 0.27	1.85 ± 2.67	< 0.001

Table 3. Frequency and type of chromosome aberrations in control and exposed group

Type of aberrations	Control	Exposed	*p* value
Chromatid type aberrations	17	42	< 0.004
Chromatid gap	14	20	
Chromatid break	3	22	
Chromosome type aberrations	15	18	< 0.870
Chromosome gap	11	8	
Chromosome break	2	4	
Acentric fragment	2	5	
Dicentric chromosome	0	1	
Ring chromosome	0		
Total number of cell with aberrations	32	60	< 0.012

group. Chromosome aberrations and comet parameters in non-smokers of the exposed worker group were significantly higher than those in the control subjects (Table 4).

Discussion

The relationship between benzene exposure and chromosome aberration has been reported previously. Zhang et al found a higher incidence of aneuploidy and long arm deletion of chromo-somes 5 and 7 in the lymphocytes of Chinese workers, who were exposed occupationally to benzene [16]. Carere et al reported a higher incidence of aneuploidy in the gasoline station attendants in Rome, Italy [3]. Smith et al reported an association between hyperdiploidy of chromosomes 8 and 21 and benzene exposure [17]. Yadav and Seth found a significant elevation of chromosome aberrations and sister chromatid exchange in petrol pump workers in India [4]. Celik and Akbas found a significant increase in the frequencies of sister chromatid exchanges and chromosome aberrations in 30 gasoline station attendants in the city of Mersin, Turkey [6]. The results in this study are in concordance with previous reports. This study found significantly higher chromosome and chromatid type aberrations in gasoline station attendants than in the unexposed control. The chromosome type aberration also was higher in the workers than in the control, but it was not statistically significant. This study found only one dicentric chromosome detected in an exposed worker. Kasuba et al detected dicentric chromosomes in both control and exposed subjects, but the incidence was significantly higher in exposed workers than in controls [18]. Anderson et al showed that benzene and its metabolites induced DNA damage in human lymphocytes demonstrated by comet assay [19]. Results from the alkaline comet assay demonstrated a highly significant increase in DNA damage to lymphocytes in gaso-

Table 4. DNA damage to non-smoking petrol pump workers and control subject

Parameters	Non-smoker (N=51)		*p* value
	Control (N=29)	Exposed (N=22)	
Tail length			
Mean ± SD (μm)	1.60 ± 1.04	5.30 ± 4.66	< 0.001[a]
Tail moment			
Mean ± SD, (μm)	0.31 ± 0.27	1.77 ± 2.45	< 0.001[a]
Percentage of cell with aberration	1.03%	1.82%	< 0.048[b]

[a] Mann-Whitney U test; [b]Chi-square test

line station attendants, when compared to the control group. The comet assay presented in this study not only confirmed the results of the chromosome aberration test, but also demonstrated a clearly significant increase in DNA damage petrol pump workers.

The exposure of these workers should be emphasized in that they were exposed at the same time to benzene other fuel components such as butadiene, toluene, and hydrocarbons that are well known mutagenic and carcinogenic agents. In addition, they weare exposed to several genotoxic substances such as airborne pollution, dust, traffic fumes, lead acetate, and carbon monoxide that were emitted from motor vehicles into the ambient air of petrol pump stations. Therefore, benzene was not the single causative agent of DNA damage in these workers.

The chromosome aberration frequencies and comet parameters between nonsmokers and smokers among petrol pump workers were not significantly different. However, there were significantly greater comet parameters and chromosome aberration frequencies found in non-smoking of petrol pump workers than in the controls. Evidence of chromosome damage was not detected in workers who had not been working for longer than six months. However, the increase in DNA damage in this subgroup was detected when using the comet assay. This finding supports previous studies that the comet assay is more sensitive than the chromosome aberration assay [11,20-22]. Among workers who had worked for a period of six months to one year, a significant increase in DNA damage was detected by using both the chromosome aberration and comet assays.

Dust particles from roads in Chiang Mai city were investigated by Vinitketkumnuen et al [23]. They collected particulate matter, PM10 and PM 2.5, in four parts of the city. The extracts were mutagenic to the Salmonella typhimurium strain, TA100, without metabolic activation. Ruenyuthikarn et al detected toxicity of PM10 collected from heavy traffic areas of Chiang Mai and Lumphun province [24]. The ethanol and acetonitrite extracts from particulate matter cause DNA damage and chromosome aberrations in human lymphocytes. These data demonstrate that the dust particles from traffic areas are genotoxic on human lymphocytes. Pollution in the petrol pump station is composed of not only gasoline and their derivatives, but also dust particles that are carried in with motor vehicles.

An increase of chromosome aberrations in circulating lymphocytes is a risk factor for contracting cancer. Forni reported that long term effects of benzene exposure on chromosome aberrations were present for up to 30 years, and the high frequency of chromosome aberrations in peripheral blood lymphocytes was related to the high risk of having neoplasia [25]. Liou et al demonstrated that in blackfoot endemic area, people who had chromosome type aberration in their lymphocytes were at higher risk of having cancer [26]. Subjects with a total frequency of chromosome type aberration of more than four had a nine fold greater risk of cancer. Hagmar et al performed a cohort study in Nordic and Italian subjects, using the chromosome aberrations test in peripheral blood lymphocytes, with a median follow-up of 17 years [27]. They found that the high level of chromosome aberrations in the individuals tested were associated clearly with an increased total incidence of cancer and cancer mortality in the Nordic and Italian cohort, respectively. Rossner et al found a significant association between the overall cancer incidence and the presence of chromosome type aberrations in the Czech Republic [28]. Subjects with a higher chromosome type aberration are at greater risk of having cancer. A report from the European collaborative project (Cancer Risk Biomarkers) indicated that both the chromatid and chromosome-type aberration predict cancer risk, even though the chromosome type may have a more pronounced predictive value [29].

It could be emphasized that petrol pump workers are at higher risk of having DNA damage and health problems. These people should take preventive measures to avoid inhaling gasoline fumes, gasoline derivatives and dust particles.

These preventive measures should be applied to all petrol pump workers worldwide.

Conclusion

This study found a significant excess of DNA damage and structural chromosome aberrations in workers who were exposed occupationally to gasoline and air pollutants, when compared to matched controls. These findings confirm the potent genotoxic effect of gasoline and air pollutants as an inducer of DNA damage and structural chromosome aberrations.

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ความผิดปกติของโครโมโซม และความเสียหายของดีเอ็นเอในผู้ที่ทำงานในปั๊มน้ำมันในจังหวัดเชียงใหม่

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วัตถุประสงค์ เพื่อสำรวจความเสียหายของดีเอ็นเอในผู้ที่ทำงานในปั๊มน้ำมันซึ่งได้รับไอระเหยของน้ำมัน อนุพันธ์ของน้ำมัน และสารปนเปื้อนในอากาศ โดยการหายใจและการสัมผัสทางผิวหนัง เปรียบเทียบกับกลุ่มควบคุม

วิธีการทดลอง ใช้การทดสอบความผิดปกติของโครโมโซมและซิงเกิลเซลล์เจลอิเล็คโทรฟอรีซิสหรือโคเมท-แอสเสย์ ประเมินสภาวะของดีเอ็นเอในเซลล์ลิมโฟไซต์ของผู้ที่ทำงานในปั๊มน้ำมัน 32 ราย จากปั๊มน้ำมัน 8 แห่งในเขตเมืองของจังหวัดเชียงใหม่ กลุ่มควบคุมจำนวน 30 ราย เป็นอาสาสมัครที่เทียบได้กับกลุ่มทดลองในด้านอายุและเพศ โดยไม่มีประวัติการประกอบอาชีพที่สัมผัสกับสารเบนซิน

ผลการทดลอง เซลล์ลิมโฟไซต์ของผู้ที่ทำงานในปั๊มน้ำมันมีความเสียหายของดีเอ็นเอมากกว่าที่พบในกลุ่มควบคุมอย่างมีนัยสำคัญ ในการทดสอบโคเม็ทแอสเสย์พบว่า tail length และ tail moment ที่วัดจาก 100 เซลล์ต่อรายของผู้ที่ทำงานในปั๊มน้ำมันมีค่าเฉลี่ยเท่ากับ 5.51±5.46 และ 1.85±2.67 μm ตามลำดับ ในขณะที่ในกลุ่มควบคุมมีค่าเฉลี่ยเท่ากับ 1.57±1.03 และ 0.31±0.27 μm ตามลำดับ ผลการตรวจความผิดปกติของโครโมโซมในเซลล์ลิมโฟไซต์ 100 เมทาเฟสต่อรายของผู้ที่ทำงานในปั๊มน้ำมันมีความถี่มากกว่าที่พบในกลุ่มควบคุมอย่างมีนัยสำคัญ เปอร์เซ็นต์เซลล์ที่มีความผิดปกติของโครโมโซมในผู้ที่ทำงานในปั๊มน้ำมันและกลุ่มควบคุมมีค่าเท่ากับ 1.87 และ 1.06 ตามลำดับ

สรุป การศึกษานี้พบว่าคนงานที่ได้รับไอระเหยของน้ำมันและสิ่งปนเปื้อนในอากาศมีความเสียหายของดีเอ็นเอมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ซึ่งยืนยันความเป็นพิษต่อยีนของน้ำมันและสิ่งปนเปื้อนในอากาศโดยชักนำให้เกิดความเสียหายของดีเอ็นเอและความผิดปกติของโครโมโซม **เชียงใหม่เวชสาร 2555; 51(1):7-13.**

คำสำคัญ: ความเสียหายของดีเอ็นเอ ความผิดปกติของโครโมโซม โคเมทแอสเสย์ ผู้ที่ทำงานในปั๊มน้ำมัน