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Lymphocyte DNA damage in Turkish asphalt workers detected by the comet assay

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Asphalt has a highly complex structure and it contains several organic compounds including polycyclic aromatic hydrocarbons and heterocyclic compounds. In this study, comet assay was used to detect the DNA damage in blood lymphocytes of 30 workers exposed to asphalt fumes and 30 nonexposed controls. This is the first report on Turkish asphalt workers' investigated DNA damage using the alkaline single cell gel electrophoresis (SCGE). The DNA damage was evaluated by the percentage of DNA in the comet tail (% tail DNA) for each cell. According to our results, workers exposed to asphalt fumes had higher DNA damage than the control group ($p < 0.01$). The present study showed that asphalt fumes caused a significant increase in DNA damage and the comet assay is a suitable method for determining DNA damage in asphalt workers.

Keywords: asphalt workers; comet assay; lymphocyte DNA damage; % tail DNA

Introduction

Occupational exposure of workers is still poorly characterized because workers perform different tasks. Asphalt workers are one of the major groups of occupational exposure because of exposure to a variety of chemical, biological, or physical (e.g. noise, heat, and radiation) agents during their working life time.

Asphalt (or bitumen) commonly used by workers in roofing or road paving operations is highly complex and contains several organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic compounds (King et al. 1984). The major route of exposure to asphalt is by inhalation, but it may also be absorbed through the skin. According to the International Agency for Research on Cancer (IARC), PAHs in the asphalt fumes are either known or suspected to be human carcinogens and are described as group three (inadequate evidence for carcinogenicity in human as of 1987) (IARC 1985). Several epidemiological studies on asphalt workers showed that occupational exposure to asphalt may increase the risk of cancers such as lung, stomach, bladder, leukemia, and nonmelanoma skin as reported by IARC (Machado et al. 1993; Partanen & Boffetta 1994; Boffetta et al. 1997; Sivak et al. 1997; Boffetta et al. 2003). However, final report from IARC, European asphalt workers exposed to asphalt

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fumes showed no consistent evidence of an association between indicators of inhalation or dermal exposure to asphalt fumes and lung cancer risk (Olsson et al. 2010). Thus, asphalt was labeled as a “high priority substance.” Furthermore, various human and animal studies can also be conducted to investigate the link between exposure to asphalt fumes and cancer. These include chromosomal aberrations, micronuclei (MN) formation, sister chromatid exchanges (SCE), and DNA damage (e.g. strand breaks, alkali-labile sites) in asphalt fume exposures (Schreiner 2010).

During the last few years, human exposure to different agents that induce DNA damage has created a growing interest in the development of new techniques such as single cell gel electrophoresis (SCGE), also known as comet assay, to identify the effects of exposure to environmental agents. Comet assay is a well-established genotoxicity test (Collins et al. 1997) and is highly effective in revealing the association between DNA damage and environmental, genetic, and acquired factors, which provides further data on the possible applicability of this assay in genotoxic human surveillance in addition to established tests (Poli et al. 1999). Even low levels of DNA damage in small numbers of cells can be detected by this assay. It is also cheap, simple, and a rapid method (Dusinska & Collins 2008; McArt et al. 2009). For these reasons, it is preferred frequently by researchers in occupational and environmental biomonitoring studies to identify DNA damage induced by exposure to in vitro and in vivo genotoxic compounds.

According to the best of our knowledge, this is the first study in the literature regarding determination of DNA damage on Turkish asphalt workers using the SCGE. The aim of the present study was to investigate genetic damage by using the comet assay in peripheral lymphocytes of the workers who are exposed to bitumen fumes.

Materials and methods

Study subjects

In the study, 30 male workers exposed to asphalt fumes (49.47 ± 5.80 years; ranging from 38 to 60 years) and 30 healthy unrelated male volunteers (48.35 ± 6.28 years; ranging from 37 to 61 years) were recruited. Blood samples of volunteers were collected in the workplace within their working time, in heparinized tubes, and stored at 4°C until the analysis. The characteristics of the study groups are presented in Table 1. Informed consent was obtained from each individual who was selected randomly from Turkish population. A small questionnaire for gathering the demographic and ethnic information was also given to the individuals, and the individuals stating themselves as Turkish were included in the study. Each of volunteers filled in detailed questionnaires regarding confounding factors for DNA damage such as smoking. The study samples comprised healthy volunteers without cancer, consumption of alcohol, chronic disease, weight-

Table 1. Characteristics of the study population.

	Control group	Exposed group
Number of subject	30	30
Age (years) (Mean \pm SD)	48.35 ± 6.28	49.47 ± 5.80
Range (years)	37–61	38–60
Smoking habits		
Smokers	8	8
Nonsmokers	22	22

reducing diet, continuous use of drugs, UV and X-ray exposure. The study design was approved by the institutional ethics committee and blood samples were handled in accordance with the principles of the Declaration of Helsinki.

Comet assay

A potential genotoxic effect of asphalt fumes was analyzed with comet assay. The comet assay was conducted under alkaline conditions with some modifications, basically as described by Singh et al. (1988). In brief, conventional microscope slides were covered with a first layer of 0.5% normal agarose. Then, a 50 μ L aliquot of the cell sample was mixed with 100 μ L of 0.5% low melting point agarose and added to the slides which were then immediately covered with coverslips. After removing the coverslips, all slides were immersed in a 4 °C lysing solution for 1 h (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, NaOH to pH 10, to which 1% Triton X-100, 1% *N*-Lauryl Sarcosine, and 10% dimethylsulfoxide [DMSO] were freshly added) in the dark. The slides were placed in an electrophoresis tank containing freshly prepared alkaline solution (300 mM NaOH, 1 mM EDTA, pH > 13), and the electrophoresis was performed for 20 min at 300 mA and 25 V. After electrophoresis, slides were taken from the tank and washed three times in neutralizing buffer for 5 min (0.4 M Tris, pH = 7.5). Afterwards, slides were fixed in a series of ethanol for 5 min. Finally, DNAs were stained with ethidium bromide (60 μ L of a 20 μ L/mL). Two slides were prepared for each sample, and randomly chosen 50 cells were measured by Comet Assay BAB Bs automatic image analysis system fitted with an Olympus BX50 fluorescence microscope. The percentage of DNA in the comet tail (% tail DNA) was used to evaluate the extent of DNA damage. % tail DNA was calculated (100 – % head DNA).

Statistical analysis

The Statistical Package for Social Sciences version 16.0 software was used for the statistical analysis. The differences between two groups were compared using the Student *t*-test or the Mann Whitney U test which is appropriate. The minimum confidence levels were chosen as 95% ($p < 0.05$).

Results

In this study, DNA damage was determined in peripheral lymphocytes of 30 asphalt workers and 30 age-matched controls by use of the alkaline comet assay. Figure 1 shows typical images of lymphocytes generated by comet in asphalt workers. As a

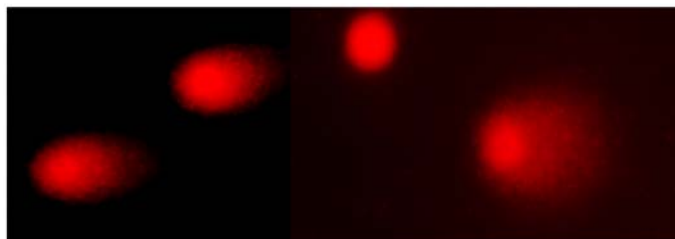


Figure 1. Typical images of lymphocytes generated by Comet 4.0 (40X magnification).

Table 2. Comparison between the % tail DNA in exposed (smoker and nonsmoker) and unexposed controls.

DNA damage	Exposed group	Control group	Exposed group	
			Smoker	Nonsmoker
% Tail DNA (Mean \pm SD)	24.34 \pm 2.72	20.04 \pm 2.75	24.82 \pm 2.53	23.00 \pm 2.96
<i>p</i>		0.001		0.108

result, increased DNA damage was found in workers exposed to asphalt fumes compared to controls. % tail DNA in asphalt fumes-exposed group was significantly higher than the control group (24.34 \pm 2.72 vs. 20.04 \pm 2.75) ($p < 0.01$).

Asphalt-exposed individuals were subdivided into two groups as smokers and nonsmokers in order to determine whether smoking increases the level of DNA damage in addition to asphalt exposure. These two groups were compared in terms of DNA damage and the results were given in Table 2. Smoker-exposed group had higher % tail DNA (24.82 \pm 2.53) than nonsmoker-exposed group (23.00 \pm 2.96), but there was not a statistically significant difference ($p > 0.05$).

Discussion

In the present study, we aimed to detect the genotoxic risk of workers exposed to asphalt fumes by the comet assay. Images were scored using the BAB Bs automatic image analysis system. This system calculated a number of comet parameters, but we only focused on % tail DNA. So far, in many studies, DNA damages have been evaluated according to different comet assay parameters by researchers. However, it has been strongly recommended that % tail DNA should be the standard parameter. This parameter is linearly related to break frequency over a wide range of damage, is relatively unaffected by threshold setting in the software, and allows discrimination of damage over the widest possible range (from 0 to 100%) (Azqueta et al. 2011; Garcia et al. 2011). % tail DNA is also considered the most reliable parameter for inter-laboratory comparisons (Kumaravel & Jha 2006). The other advantage of % tail DNA is a scale-independent parameter giving a clear indication of comet appearance (Garcia et al. 2011). Kumaravel and Jha (2006) reported that % tail DNA is more meaningful and easy to conceptualize than other comet parameters in genotoxicological studies (Kumaravel & Jha 2006).

There have been conflicting insights in literature with regards to whether *N*-lauryl sarcosine and DMSO should be present in lysis or not (Hartmann et al. 2003; Yan & Galdwell 2004). DMSO was added as a free radical scavenger to prevent potential radical-induced DNA damage associated with the iron released during lysis from erythrocytes present in blood and tissue samples containing heme (Hartmann et al. 2003). In order to complete the lysis, 1% *N*-lauryl sarcosine was used as a second detergent (Yan & Galdwell 2004).

Asphalts have been tested to show mutagenic/genotoxic effects of asphalt fumes in a range of in vitro and in vivo animal or worker monitoring studies (Qian et al. 1996; Qian et al. 1999; Reinke et al. 2000; Toraason et al. 2001; Ma et al. 2002; Zhao et al. 2004; Halter et al. 2007; Schreiner 2010). Asphalt workers showed an elevated level of DNA strand-breaks and oxidative DNA damage in some studies using comet assay (Toraason et al. 2001; Cavallo et al. 2006; Marczynski et al. 2006, 2011; Lindberg

et al. 2008). In the present study, we found that the workers exposed to asphalt fumes had significantly higher DNA damage than the controls. Thus, our result is in line with the previous studies in which asphalt workers showed a significantly higher level of DNA single-strand breaks (Cavallo et al. 2006; Marczynski et al. 2006, 2011). To our knowledge, this is the first study to detect DNA damage in Turkish workers exposed to asphalt fumes by using comet assay.

Other types of DNA damage have also been evaluated in previous studies of asphalt workers. The possible genotoxic effects induced by exposure to asphalt fumes are SCE and MN formation in exposed workers (Burgaz et al. 1998; Karaman & Pirim 2009). Murray and Edwards showed that MN formation in asphalt-exposed workers was higher than in controls (Murray & Edwards 2005). In a study of workers exposed to asphalt fumes, DNA strand breaks were higher in blood lymphocytes from bitumen-exposed workers before and after shift compared with the reference group (Marczynski et al. 2006, 2007, 2011). Contrasting results were shown in studies of road pavers exposed to asphalt fumes. There were not significant increases in MN formation and/or SCE in peripheral lymphocytes from road pavers in some studies (Järholm et al. 1999; Cavallo et al. 2006). In the study performed by Lindberg et al., there was not a statistically significant difference in DNA strand-break level of buccal leukocytes between the pre-and post-shift exposed workers (Lindberg et al. 2008).

Tobacco smoking is shown to be associated with DNA damage in some studies (Sram & Binkova 2000; Söylemez et al. 2012). In consistent with these studies, we found that the smoker-exposed group had higher % tail DNA than the nonsmoker exposed group; but this result was not statistically significant ($p > 0.05$).

In conclusion, our results showed that exposure to asphalt fumes have genotoxic effect on peripheral lymphocytes, measured by comet assay, suggesting a possible association between occupational exposure in asphalt workers and its genotoxic effect. Future studies including various biomarkers of DNA damage may help characterizing the occupational exposure and estimating the effects of asphalt fumes to health.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgment

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