

Evaluation of Relationship Between Lymphocyte DNA Damages and Blood Arsenic Levels in Silver Mining Workers using Alkaline Comet Assay

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ABSTRACT

The purpose of this study is to investigate the association between DNA damage and blood arsenic levels in individuals occupationally exposed to arsenic (As). A hundred-twenty exposed individuals from silver mining plate workers were monitored in the way of DNA damage in blood lymphocytes using comet assay (single cell gel electrophoresis, SSGE). The DNA damage levels were measured by BAB Bs Comet Assay system and As levels were analyzed by the atomic absorption

spectrometer (AAS) system. The mean blood arsenic level was 17.03 ± 10.85 µg/L. A statistically significant positive correlations were found between the arsenic levels and comet parameters (tail intensity, comet length and tail length) ($r=0.360$, $r=0.334$, $r=0.259$, respectively, $p<0.01$). Our results showed that exposure to arsenic may cause DNA damage in peripheral lymphocytes of Turkish silver mining plate workers.

Keywords: DNA damage; blood arsenic levels; Turkish workers; comet assay.

Introduction

Arsenic is one of the most widely studied elements in the field of metal intoxication and it is a well-known reactive oxygen species (ROS) inducer. Chronic exposure to arsenic compounds has been associated with different types of cancer such as skin, scrotal, liver, lymphatic system, and lung, and several non-cancer health effects (diabetes, many cardiovascular and neurological diseases) (1-3). The International Agency for Research on Cancer (IARC) classified inorganic As in Group 1 human carcinogen (4). Arsenic toxicity depends on its oxidation state, solubility and concentration of different inorganic and organic arsenic compounds. Arsine is considered to be the most toxic form followed by arsenite (AsIII), arsenate (AsV) and organic arsenic compounds (5). Inorganic trivalent arsenite is 2 –10 times more toxic than pentavalent arsenate; and dimethyl arsenic acid (DMAA3) and monomethyl arsenic acid (MMAA3) are more toxic than their main components. It has been reported that arsenic compounds induce DNA damage in human lymphocytes (6). Arsenic toxicity can occur directly through arsenic-thiol enzyme interactions and indirectly through ROS-enzyme interactions which generate DNA adducts, single DNA strand breaks, sister chromatid exchanges, micronucleus formation and chromosomal

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aberrations (7-9). It has also been shown that arsenic can induce DNA damage in multiple test systems (10).

The alkaline single cell gel electrophoresis (SCGE) technique, also known as comet assay, is a well-established genotoxicity test. SCGE is highly effective for revealing the association between DNA damage and environmental, genetic, and acquired factors. It also provides further data on the possible applicability in genotoxic human surveillance in addition to established tests (7, 11, 12). The comet assay is based on the ability of negatively charged fragments of DNA that are drawn through an agarose gel in response to an electric field. The extent of DNA migration depends directly on the DNA damage in the cells. This assay can be used to estimate DNA damage at the individual cell level by identifying single and double-strand DNA breaks, DNA-DNA/DNA-protein cross-links, oxidative base damage, alkali-labile sites and open repair sites (7, 13). It is applied to both in vivo and in vitro studies for many cells. SCGE is a simple, sensitive, fast and effective method for analyzing the cell samples from any organ of eukaryotic organisms in small quantities. Thus, it has been widely used for the studies in genetic toxicology, medical research and radiation biology and DNA repair studies, environmental and human biomonitoring studies (12, 14). Currently, DNA damage detected by the comet assay is considered to be an early indicator of genetic disease or cancer risk. It is also an indicator of exposure to a wide variety of genotoxic agents, and a sensitive endpoint for detecting DNA damage (12, 15, 16). Thus, this assay is an ideal biomonitoring technique to evaluate the genotoxicity of arsenic in lymphocytes of exposed individuals (7).

In the literature, there are several studies concerning the genotoxic effects of arsenic that was detected by comet assay in cell culture (17-19), rats (20, 21), and children (22). There have also been some studies evaluating the effects occupational arsenic exposure on DNA damage using comet assay (23-25). However, those studies used only one comet parameter to detect DNA damage and the study subjects were chosen from glass factory workers and copper smelters. The present study was the first study evaluating the genotoxic effects of arsenic by three comet assay parameters in Turkish silver mining plate workers.

Materials and Methods

Study Subjects

The study population comprised 120 Turkish silver mining plate workers who stated themselves as Turkish. A small

questionnaire for gathering the information including age, exposure time and smoking habit was given to the individuals. The study design was approved by the institutional ethics committee (approval number: 06-242-13). Written informed consent was obtained from each worker. Samplings were performed in accordance with the principles of The Declaration of Helsinki. Blood samples collected into tubes with heparin for comet assay and into tubes with EDTA for metal analysis.

Comet assay

DNA damage was determined according to tail intensity (TI), comet length (CL) and tail length (TL) comet parameters (13). The levels of DNA damage were measured by the BAB Bs Comet Assay system.

The comet assay was conducted under alkaline conditions with some modifications, essentially as described by Singh *et al* 1988 (26). In brief, conventional microscope slides were first covered with a layer of 0.5% normal agarose. Then, a 50 μ L aliquot of the cell sample was mixed with 100 μ L 0.5% low-melting-point agarose and added to the slides, which were then immediately covered with coverslips. After removing the coverslips, all of the slides were immersed in a 4°C lysing solution (2.5 M NaCl; 100 mM EDTA; 10 mM Tris; and NaOH, pH 10, to which 1% Triton X-100, 1% *N*-lauryl sarcosine, and 10% DMSO were freshly added) for 1 hour 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 electrophoresis was performed for 20 minutes at 300 mA and 25 V. After electrophoresis, the slides were taken from the tank and washed three times in neutralising buffer for 5 minutes (0.4 M Tris, pH 7.5). Afterwards, the slides were fixed in an ethanol series for 5 minutes. Finally, DNA was stained with ethidium bromide (60 μ L of a 20 μ L/mL). Two slides were prepared for each sample, and 100 randomly chosen cells were measured by comet assay BAB Bs automatic image analysis system fitted with an Olympus BX50 fluorescence microscope (Figure 1). All steps were conducted in the dark to prevent additional DNA damage (13).

Determination of blood arsenic levels

A microwave system (CEM Mars Xpress) was utilised for the digestion of blood samples with concentrated nitric acid solution. Analysis was performed with a dual atomic absorption spectrophotometer (AAS) system (Varian 240). A

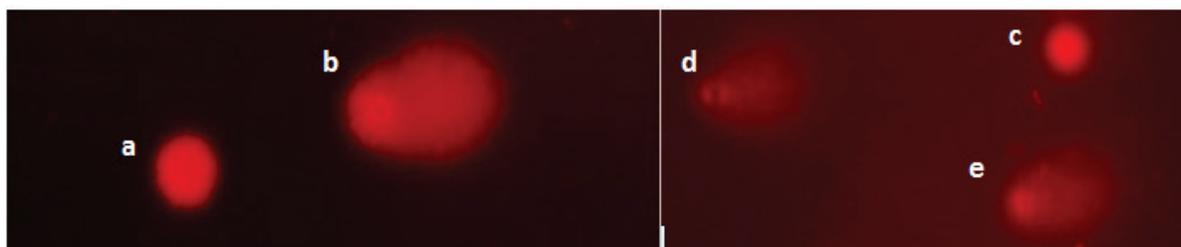


Figure 1. Representative comet assays of cells (a and c: Undamaged cells; b, d, e, f: damaged cells)

total of 1 ml of the whole blood sample was dissolved in 10 ml nitric acid, after which all of the samples were transferred to Teflon tubes and digested in a microwave at 200°C for 20 min. The digested sample solutions were diluted before being introduced into AAS equipped with a graphite furnace and Zeeman background correction system. The AAS method was evaluated by comparing the certified reference materials (Serorm TM Trace Elements Whole Blood Level-2; Ref Number: 201605) with the certified values.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 16.0 software for Windows was used for the statistical analysis. Continuous variables are reported as means±standard deviation (S.D.) and compared by the Student t-test for two groups. Categorical variables were compared by the χ^2 test. The Pearson correlation coefficient was used for determination of the association between two variables. Results were considered to be statistically significant at $p < 0.05$.

Results

The general information of workers' age, exposure time, comet parameters and blood arsenic levels were given in Table 1. The mean age of the workers was 32.58 ± 8.02 years. The mean values of the TI, CL, and TL were 429097.25 ± 213775.29 , 27.23 ± 3.05 and 5.30 ± 0.85 , respectively. The mean blood arsenic level of the workers was 17.03 ± 10.85 $\mu\text{g/L}$.

When the correlation coefficients of the comet parameters, blood arsenic levels and length of employment were calculated, a statistically significant positive correlation was found between the blood arsenic levels and TI, CL, and TL parameters ($r = 0.360$, $p = 0.001$; $r = 0.334$, $p = 0.001$; $r = 0.259$, $p = 0.004$, respectively). The length of employment was found to be not correlated with blood arsenic levels and comet parameters ($p > 0.05$). Table 2 shows the correlation coefficients between blood arsenic levels, comet parameters and the length of employment.

To detect the effect of smoking on blood arsenic levels and DNA damage, workers were also grouped into two according to their smoking habits; smokers ($n = 75$) and

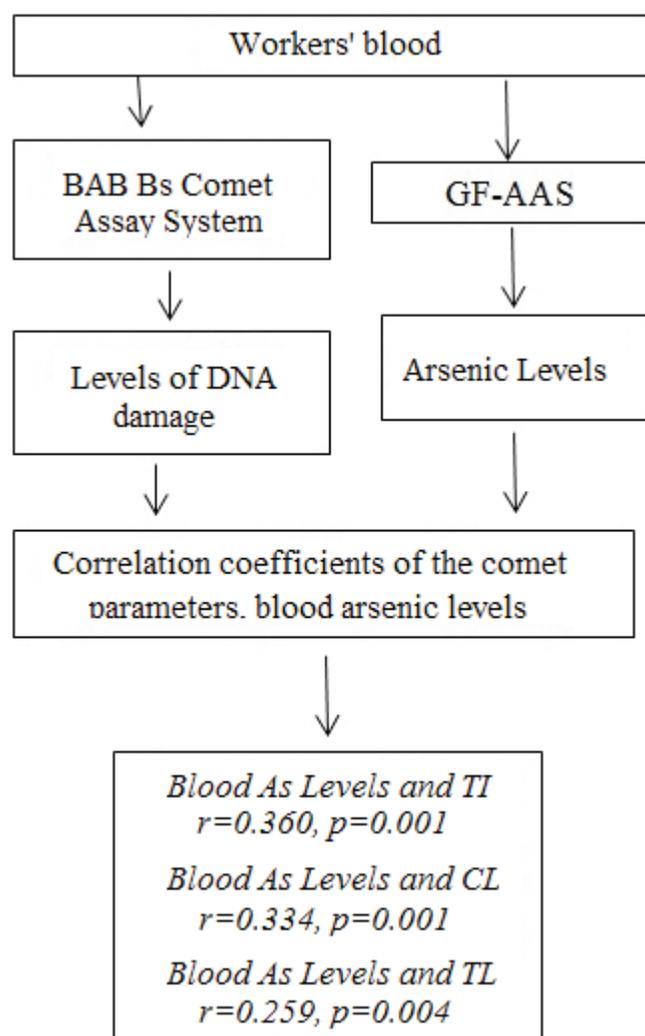
Table 1. General information of workers' age, comet parameters, blood arsenic level and the length of employment.

Parameters	Mean±S.D.*	Minimum	Maximum
Age (years)	32.58 ± 8.02	18	61
TI	429097.25 ± 213775.29	30109.03	882390.31
CL	27.2340 ± 3.04757	10.89	36.28
TL	5.3042 ± 0.84694	2.04	7.66
Blood arsenic level ($\mu\text{g/L}$)	17.03 ± 10.85	3.52	52.44
The length of employment (years)	3.31 ± 1.99	1	7

*S.D.: Standard deviation

Table 2. Correlations between comet parameters (TI, CL, TL) and blood arsenic levels.

Parameters	TI	CL	TL
TI	r=1.000		
CL	r=0.586* p=0.001	r= 1.000	
TL	r=0.554* p=0.001	r=0.826* p=0.001	r= 1.000
Blood arsenic level ($\mu\text{g/L}$)	r=0.360* p=0.001	r=0.334* p=0.001	r=0.259* p=0.004

*significance $p < 0.01$ **Figure 2.** An illustrative overview scheme describing the experimental set-up and the significant results.

non-smokers (n=45). Both the mean blood arsenic levels and comet parameters were found higher in smokers than those in non-smokers, but this was not statistically significant ($p > 0.05$) (Table 3).

A schematic picture describing the experimental set-up and the results was shown in Figure 2.

Discussion

Occupational exposure to arsenic may occur in several industries such as refining or smelting of metal ores, microelectronics, wood preservation, wood joinery shops, battery manufacturing, and working in power plants (6). In the present study, the mean blood arsenic level was $17.03 \pm 10.85 \mu\text{g/L}$, indicating that silver mining plate workers may expose to arsenic occupationally.

Arsenic has long been known as a carcinogen (27, 28). Chronic arsenic exposure adversely affects different multi organ systems such as skin, respiratory, cardiovascular, immune, genitourinary, reproductive, gastrointestinal and nervous systems of human body (2-3). Epidemiological studies have indicated that workers can be exposed to arsenic compounds via inhalation, ingestion, dermal contact, and the parenteral route to some extent. These exposure pathways increase cancer risk of lung, skin, liver, kidney, lung, colon, bladder and the lymphatic system (28, 29). Arsenic can exert its carcinogenic effect indirectly through ROS-enzyme interactions generating DNA damages such as DNA adducts, single DNA strand-breaks, sister chromatid exchanges and chromosomal aberrations which are related

Table 3. Comparison of comet parameters and blood arsenic levels between smokers and non-smokers.

Smoking habit	n	TI mean±S.D.*	CL mean±S.D.*	TL mean±S.D.*	Arsenic level (µg/L) mean±S.D.*
Smokers	75	433645.35±2.00	27.32±2.61	5.34±0.89	17.24±10.96
Non-smokers	45	426368.38±2.23	27.18±3.30	5.24±0.76	16.69±10.77
p		0.858	0.806	0.546	0.791

*S.D.: Standard deviation

to carcinogenesis (2, 30). In the present study and the previous study, there was a significant correlation between blood arsenic levels and comet parameters, suggesting that occupational arsenic exposure may cause lymphocyte DNA damage (31). These findings were consistent with previous studies using workers who exposed to arsenic (23, 24). This study is also compatible with several studies that arsenic causes genotoxic effects, chromosomal abnormality, sister chromatid exchange, DNA strand breakage, oxidative DNA damage (7, 23,27). Studies carried on individuals who have been exposed to various chemicals and toxicants at different occupational settings have shown that DNA damage level increases along with occupational exposure (16, 32-36). In contrary to these studies, we could not find a statistically significant difference between exposure time and DNA damage. We thought that this may be due to the short length of employment (approximately 3 years).

Workers were also grouped according to their smoking habits. Smokers and non-smokers were compared in view of blood arsenic levels and comet parameters (TI, CL, TL). Smokers were found to have higher blood arsenic levels and DNA damage levels than non-smokers. However, there was not a statistically significant difference ($p>0.05$). Various studies have achieved conflicting results about whether smoking causes lymphocyte DNA damage. While several studies express that smoking causes DNA damage (34, 36), some other studies report that smoking does not affect DNA damage (37, 39). There are also studies showing the combined effect of cigarette smoke and arsenic on DNA damage (42), the risk of lung cancer (42-44) and skin lesions (45). In conclusion, occupational arsenic exposure may induce DNA damage in Turkish silver mining plate

workers. In order to decrease health hazards, especially arsenic-induced cancers, we suggest that arsenic exposure should be minimized in workplaces with preventive precautions.

In conclusion; occupational exposure studies about genotoxicity investigations of individuals exposed to As are insufficient and there is not any such as study in Turkey; We think that our study will make contribution to the literature. According to our study's results it's being seen that these workers have been significantly exposed to Arsenic and this exposure caused DNA damage. The increasing of DNA damage as the As level increases in blood, strengthens carcinogenicity and may increase individuals' possibility to get cancer. Increase of As level in blood and correspondingly DNA damage in a short time may denote that exposure may be intense or that repair enzymes of individual are defected. In our next study it is going to be investigated that if the damage is originated from DNA repair enzymes being polymorphic or not; and individual count is going to be increased. Also with this study it is being seen that safer work environments shall be provided considering workers' exposure and health. This is a study that shows environments which have these kinds of toxic and carcinogens are facing with what kind of a threat.

Disclosure summary

The authors have nothing to declare.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Gümüş Madeni İşçilerinde Kan Arsenik Düzeyi ve Lenfosit DNA hasarı Arasındaki İlişkinin Alkali Kometa Analizi ile Değerlendirilmesi

ÖZ

Bu çalışmadaki amaç, mesleki arseniğe maruz bireylerde kan arsenik (As) düzeyi ve DNA hasarı arasındaki ilişkiyi araştırmaktır. Gümüş madeni işçilerinden 120 bireyin lenfositlerindeki DNA hasarı comet yöntemi (tek hücre jel elektroforezi) kullanılarak belirlendi. DNA hasarı düzeyleri BAB Bs Comet Assay sistemi ile hesaplandı ve As düzeyleri atomic

absorbsiyon spektroskopisi (AAS) cihazı ile analiz edildi. Kan As düzeyi ortalaması 17.03 ± 10.85 $\mu\text{g/L}$ olarak tespit edildi. Kan As ve comet parametreleri (kuyruk yoğunluğu, comet uzunluğu ve kuyruk uzunluğu) arasında istatistiksel olarak anlamlı pozitif korelasyon bulundu (sırasıyla; $r=0.360$, $r=0.334$, $r=0.259$; $p<0.01$). Sonuçlarımız, Türk Eti Maden gümüş işçilerinde arsenik maruziyetinin periferik lenfosit DNA hasarına sebep olduğunu gösterdi.

Anahtar kelimeler: DNA hasarı, kan arsenik düzeyi, Türk işçiler, comet yöntemi

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