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Detection of DNA damage in lead (Pb) exposed city traffic wardens in Pakistan

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Lead (Pb) is one of the toxic metals and is commonly used in industries. It affects multiple systems and has role in the production of reactive oxygen species (ROS). The objective of the present study was to determine Pb levels and to detect DNA damage in traffic wardens of Lahore, Pakistan. A total of 90 subjects were selected including 60 traffic wardens working in field and 30 working in administrative zones. The wardens who were working in field were considered as cases (exposed by the lead polluted environment) while the wardens in the administrative offices (less exposed to polluted environment) were labeled controls. Venous blood samples were collected for Pb and comet assay. In cases, the levels of Pb were 18.76±8.84 µg/dL (Mean±SD) that was higher than controls, 12.00±3.552 (*p* 0.000). Tail moment (TM) in cases (0.583±1.960) and controls (0.0453±0.108) significantly differed (*p* 0.004). There was no change in %DNA in tail and head (*p* 0.136). The parameters for DNA damage assessment including Comet length (CL) and Tail length (TL) were also found higher in cases than controls. Correlation of lead with other biochemical parameters including liver functions, renal functions and lipid profiles was carried out to assess the various organs/systems. The correlation was established with creatinine showing statistically significant value (*p* 0.019). Our findings elaborate a mild raise of lead levels in exposed group. There was no significant difference in comet length between cases and controls. Also, minor or no differences were observed in different biochemical parameters between cases and controls. These findings demonstrate dramatic improvement over the results from the study done previously in 2005 and may be attributed to the success of having lead-free petroleum as motor fuel.

Key words: Lead, DNA damage, single cell gel electrophoresis, traffic wardens.

INTRODUCTION

Lead (Pb) is one of the most useful metals having its applications worldwide but it is among the most toxic metals (Shotyk and Le Roux, 2005). The heavy use of Pb in industries, in water, in urban polluted air has caused

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global contamination of air, water and soil. Multiple researches find that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies (Patrick, 2006). Lead exhibits various biochemical effects, including enzyme inhibition, DNA damage, mutation, chromosome aberrations. It is also proven to be carcinogenic and produce congenital deformities (Johnson, 1998).

DNA damages are physical abnormalities in DNA such as single and double strand breaks, 8-hydroxyguanosine or polycyclic aromatic adducts. Oxidative DNA damage caused by reactive oxygen species involves single or double stranded DNA breaks (SSB, DSB), purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. DNA damages are recognized usually by enzymes and can be corrected if any information such as the undamaged sequence in a complementary DNA strand or in a homologous chromosome is available for copying. If a cell retains DNA damage, transcription of gene can be prevented and, therefore, translation into a protein will also be halted as a result replication will be blocked and may lead to genomic disturbances, carcinogenesis and cell death (Best, 2009; Marnett, 2000). According to World Health Organization (WHO) and The Centers for Disease Control and Prevention (CDC) of the United States Department of Health and Human Services, blood lead level (BLL) of more than 10 µg/dL is an indicator of significant Pb exposure (Binns et al., 2007). There are three main categories of agents which induce DNA damages including; a) environmental agents such as ultraviolet (UV) light (Khisroon et al., 2015), ionizing radiations (Oyar et al., 2012) and various genotoxic chemical producing DNA structural alterations, b) products of normal cellular metabolism which creates an ongoing source of DNA damage, and c) certain chemical agents like aromatic compounds and chemotherapy agents (Mah et al., 2010) which bind to DNA causing its disintegration under normal physiological conditions (Liao et al., 2009).

A cell that has accumulated large amount of DNA damage can have three possible fates. First is an irreversible state of dormancy, known as senescence. Second, can undergo apoptosis or programmed cell death. Third, it may undergo unregulated cell division which can lead to the formation of tumor that is cancerous (Browner et al., 2004). The four types of pathways elicited by DNA damage assumed to reorganize harmful damage effects are DNA repair, DNA damage checkpoints, transcriptional response, and apoptosis. Defects in any of these pathways may cause genomic instability (Khisroon et al., 2015; Sancar et al., 2004).

A study in Pakistan Islamabad showed that traffic constables have significantly higher Pb levels than the normal population. Also, the Pb levels of the officers of Karachi traffic police are much higher than that of the traffic policemen of Islamabad which is attributable to increase in traffic (Agha et al., 2005). The purpose of this study was to check the levels of the Pb along with its effect on DNA in city traffic wardens of Lahore and Lahore metropolitan after the introduction of unleaded petrol, because these traffic wardens are occupationally exposed to Pb pollution and no studies have been done so far in this particular group.

MATERIALS AND METHODS

Prior to start of the study, permission was granted from Ethical Review Committee, University of Health Sciences, Lahore, Pakistan and informed consent was obtained from all participants. Guidelines of Helsinki (2013) were followed for human analysis.

Population selection

A total of 90 traffic wardens including 60 traffic wardens working in field and 30 wardens involved for administrative work were selected to execute the present study. The wardens who were working in field (at least for one year) were considered to expose the lead polluted environment (cases) while the wardens in the administrative offices were labeled as un-exposed to lead (controls). The wardens who were known for any chronic disease, autoimmune disorders and smokers were excluded from this study. Control group consisted of healthy office workers and students who had never been occupationally exposed to known genotoxic agent.

Sample collection

After an informed consent, blood samples were collected from all participants by aseptic technique. A 5 ml blood was obtained and equally divided into 2 tubes. For cell extraction 2.5 mL of heparanized blood was collected in heparin containing vials and mixed properly and 2.5mL of blood sample was transferred in black top metal free nitric acid treated tube. Cell extraction was performed from heparanized blood and serum was separated from nitric acid containing tubes. Cells were processed for comet assay and serum for Pb analysis.

Analysis of lead (Pb)

Blood sample was prepared for Pb analysis by the method of Subramanian and Jeans (Subramanian and Meares, 1985). Serum was digested with 10% nitric acid and then it was diluted by 1:5, in 10% nitric acid. Pb was analyzed using Atomic absorption spectrophotometer with graphite furnace (Hitachi Z2000, AAS). Procedure was calibrated by using multi-calibrate solution (Merck). Argon gas was used to provide an inert environment.

Determination of DNA damage

DNA damage was detected by comet assay technique also referred to as single cell gel electrophoresis assay (SCGE). In this method, lymphocytes (leukocytes) were isolated from buffy coat by ficol method (Lymphocyte Separation Medium, MP Biomedicals, Inc, USA). Cells were washed by phosphate buffer saline (PBS, pH8.0, 2 mM). Lymphocytes were counted by Neubar chamber (heamocytometer) and equivalent to 50,000 cells was considered for further processing. Cells were stored in PBS (pH 8.0) at 4°C overnight (Khisroon et al., 2015). On the next day, the slides were prepared by suspending cells in low melting point agarose (LMPA;

Table 1. Comparison of different parameters in traffic wardens and controls.

Parameter	Mean ± S.D (Case)	Mean ± S.D (Control)	*p*-value
Age	27.62±2.148	28.13±2.330	0.241
Blood lead level (*µ*g/dL)	18.76 ±8.84	12.00±3.552	0.000*****
Tail moment (µm)	0.583±1.960	0.0453±0.108	0.004*****
%DNA in tail	4.100±5.871	2.055±2.481	0.136
%DNA in head	95.90±5.871	97.94±2.481	0.136
Comet length (µm)	123.7±36.7	99.56±31.81	0.003*****
Tail length (µm)	7.516±12.56	1.500±2.129	0.003*****

*****p value <0.05 is considered to be statistically significant.

Table 2. Correlation of Lead (Pb) levels with parameters of DNA damage.

Lead correlation with (*µ*g/dL)	Correlation coefficient (rho)		*p*-value	
	Case	Control	Case	Control
Tail moment (µm)	0.337	0.407	0.008*	0.026*
%DNA in tail	0.018%	+ 0.380%	0.003*	0.926
%DNA in head	-0.380%	+0.380%	0.003*	0.926
Comet length (µm)	0.134	0.191	0.306	0.313
Tail length (µm)	0.185	0.226	0.15	0.231

*****p value 0f <0.05 is considered to be statistically significant.

melt at 65.5ºC). Then unwinding of DNA was done by using lysis solution (2.5 M NaCl 100 mM, EDTA 10 mM, Tris pH10, 1 % triton x 100 and 10% DMSO). After unwinding, the slides were kept in alkaline buffer (1 mM EDTA, 300 mM NaOH pH13.0) to produce single stranded DNA. After that, electrophoresis was run (25 V and 300 mA) for 20 minutes. Neutralization was done by buffer (0.4 M Tris pH 7.0). Fixation of slides was carried out in 30% methanol. At the end, the slides were stained by DAPI (6-diamidino- 2-phenylindole) and scoring was done by comparing the standard measurement like percentage of DNA in the tail, tail length measured from the center of the comet head that was calculated as the tail length multiplied by the percentage of DNA in the tail and by tail moment (TM). DNA damage was considered when a comet noted with a distinct head consisting of intact DNA and a tail which contains damaged or broken pieces of DNA, on visualizing by fluorescent microscope. Images were captured using Charge-coupled device (CCD) camera attached with the microscope.

RESULTS

In the present study, a total of 60 traffic wardens from different zones of Lahore city were investigated along with 30 controls wardens.

Table 1 represents the comparison of different variables tested in traffic wardens and controls. Lead (Pb) levels were found higher in cases with concentration of 18.76±8.84 (Mean±SD) with a range of 2-44 µg/dL while the levels of Pb were 12.00±3.552 (p 0.000). For DNA damage assessment, Tail moment (TM) was altered in cases (Mean±SD 0.583±1.960) as compared to the controls (Mean±S.D 0.0453±0.108) showing statistically significant difference (p 0.004). The percentage of DNA in tail and head showed no significant difference (p 0.136). The range of comet length (CL) in cases was 48.0 to 237.0 µm and from 48.0 to 166.0 µm in controls reported statistically significant difference (p 0.003). Tail length (TL) was from 0.000-79.00 µm in cases and from 0.00 to 18.00 µm in controls with a statistically difference in their means (p 0.003).

Table 2 represents the correlation of different variables of DNA damage with Pb. There was statistically significant correlation of Pb with TM, %DNA in head and in tail only in cases. There was no correlation of Pb with TL and CL in both cases in controls and controls. Table 3 shows the correlation of lead with other parameters including liver functions, renal functions and lipid profile but the correlation was documented with creatinine (p 0.019) Figures 1 and 2 demonstrated the migration pattern of comets in cases and controls and cellular pattern was more betrayed in cases.

DISCUSSION

Air pollutants generated from traffic and industrial plants are believed to be one of the major causes of DNA damage in living species. As a result of rapid urbanization, air pollution and environmental quality deterioration our daily lives have been affected including as well as the nature. Under these circumstances, humans, plants and animals might suffer from various damages. Traffic policemen are heavily exposed to vehicle exhausts

Table 3. Correlation of blood lead levels with biochemical parameters.

Parameter	Correlation coefficient	*p-value*
Creatinine (mg/dl)	0.235	0.019*
ALT (U/L)	0.007	0.943
AST (U/L)	0.063	0.563
Uric Acid (mg/dl)	0.050	0.623
Heamoglobin (gm/dl)	-0.19	0.854
Cholesterol (mg/dl)	-0.101	0.319

p value of < 0.05 was considered statistically significant.

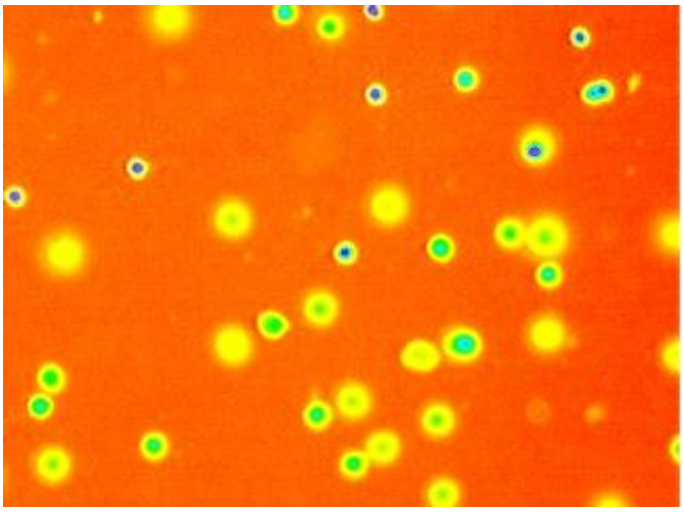

Figure 1. Assesment of DNA damage in controls of traffic wardens. Cells are perfectly round and there is no migration of DNA from nucleus.

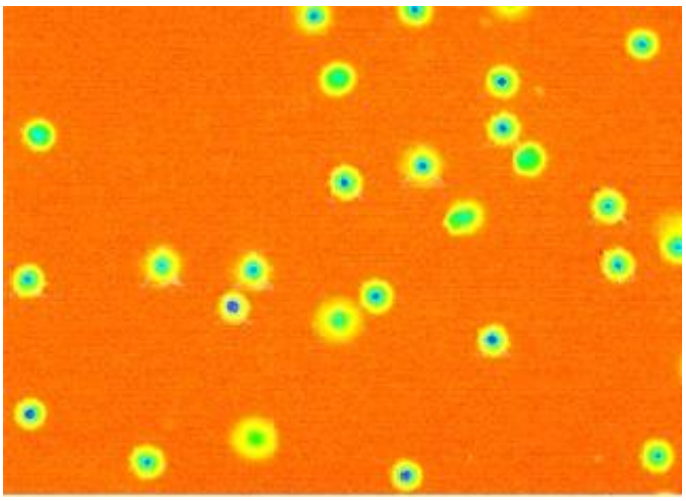

Figure 2. Assesment of DNA damage in cases of traffic wardens. The figure shows that there is more haziness and spikes in the lymphocytes of traffic wardens who are exposed to the petroleum polluted environment, although, the difference in comparison to controls is minor.

during traffic control and other outdoor activities.

Our study reveals a significant increased in the blood lead levels in traffic wardens. The result was less than the previous studies from Pakistan documented higher Pb levels in wardens (Agha et al., 2005; Sadaruddin et al., 1995). Another study in Alexandria, Egypt reported that their traffic constables had a higher blood Pb level than our study (Zaki et al., 1998).The higher levels of Pb in Egypt and Karachi might be due to increased industrialization and urbanization. Also, another reason can be due to implementation of law of unleaded petroleum decreased the Pb levels in contrast to previous studies. In a comparable study from Nigeria, the Pb level was high in wardens than controls (Ogunsola et al., 1994). Higher concentrations of Pb in body have been documented to induce cellular DNA damage (Danadevi et al., 2003).

In current study, various parameters of DNA damage were investigated by comet assay. In lead exposed group, the mean tail moment (TM), % DNA in tail and mean (Mean±S.D) tail length (TL) were 0.583±1.960, 4.101±5.87 and 7.156±12.56 respectively. Evaluation of biochemical parameters showed significant increase in creatinine levels ($p<0.05$) in traffic police wardens of Lahore. Renal system is one of the most sensitive targets for lead toxicity small increase in blood lead levels can affect renal system (Rosin, 2009). The results of present study supported this view of lead toxicity.

As documented in the earlier studies, chronic lead exposure also significantly affects other biochemical parameters like ALT, AST, cholesterol, uric acid and heamoglobin (Shotyk and Le Roux, 2005). Although, in the present study, no significant correlation was observed among blood lead levels and the biochemical parameters which are investigated. It might be due to the mean lead levels were not too high to affect these systems.

In controls (un-exposed to lead), the average mean (Mean±S.D) of TM was 0.045±0.108, %DNA in tail was 2.055±2.481 and TL was 1.50±2.12. TM and TL were statistically significant in cases and controls (p <0.05) but %DNA in tail was insignificant. There was a statistically significant correlation of Pb with TM, but no correlation with TL in cases. The other parameter was %DNA in head with an insignificant difference in the mean value of cases and controls (p 0.136). There was a statistically significant correlation of Pb with TM, but no correlation with TL in cases. There was a mild correlation of Pb with %DNA in tail in lead exposed cases. The %DNA in head showed a mild negative correlation with Pb in cases (p 0.03 and *rho* -0.380). Comet length (CL) was statistically significant (p 0.003) in cases and controls but no correlation was found with Pb (p >0.05).

A research group from Poland described the extent of DNA damage in lead exposed category. DNA strand breaks (%DNA in tail, TL, TM) were significantly associated with higher concentration of Pb in exposed workers than in unexposed (Olewinska et al., 2010). However, TL positively correlated with blood Pb level contradicting with the present study. TL has been widely used in bio-monitoring studies, although it has been criticized due to sensitivity to the background or threshold setting of the image analysis program. Genotxic effects of Pb have been also mentioned in an investigation from China (Chen et al., 2006).

Apart from above described studies, some other data has been represented to clarify the *in vivo* mechanisms responsible for the effects observed in the comet assay in lymphocytes of battery plant workers (Fracasso et al., 2002). In a study conducted by Valverde and colleagues (2002), a Pb inhalation model in mice was used to detect the induction of genotoxic damage as single-strand breaks and alkali-labile sites in several mouse organs (nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow, brain, and testes), assessed by the comet assay (Valverde et al., 2002; Bohr, 2002). Following single and subsequent inhalations, differences were found among the organs studied.

Studies carried out in wardens in various countries showed that Pb was increased as compared to controls. Similar results are being represented in the present study. According to the published data, it is the first report from Pakistan highlighting the connection of blood Pb level and DNA damage in traffic wardens. Though compared to controls the Pb level was higher in study group but it was within normal limits, indicating that lead in the atmosphere is not much to induce toxicity. This study also showed that the use of unleaded petroleum in vehicles might have contributed to decrease in level of Pb from the atmosphere that is in fact an important step taken by the Government regarding the health of citizens. Follow up studies should be done to see whether long term exposure increases the lead level or not.

Conclusion

Our findings elaborate a mild raise of Lead levels in exposed group. There was no significant difference in comet length between cases and controls. Also minor or non significant differences were observed in different biochemical parameters between cases and controls. These findings show a significant improvement in status compared to the results from study done a decade earlier and may be attributed to the introduction of lead-free petroleum in Pakistan a decade ago. These results highlight the importance of such reports for the identifying and managing exposure to environmental pollutants.

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Conflict of interest

All authors declare no conflict of interest.

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