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ORIGINAL ARTICLE

Effect of Lead Toxicity and its Correlation with Different Variables of DNA Damage

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ABSTRACT

Objective: To establish correlations of blood lead levels with DNA damage in population frequently exposed to lead in urban city.

Patients and Methods: This cross-sectional study was carried out in 60 traffic police wardens working in nine different administrative towns of Lahore. Cases were selected according to inclusion and exclusion criteria through stratified random sampling using proportional allocation. Total 30 healthy controls were taken from University of Health Sciences. Blood samples were drawn after informed consent. Blood lead concentration was determined by atomic absorption spectrometer and DNA damage by comet assay method. Data analysis was done by SPSS version 18. Correlation between lead level and DNA damage was studied.

Results: Police wardens working in open environment had significantly higher mean values of blood lead. Mean serum lead levels in study group exposed to lead were significantly elevated as compared with control group. Their blood lead levels were correlated with different variables of DNA damage.

Conclusion: As a result of industrial development, individuals exposed to lead due to their professional work, had significantly higher mean values of blood lead.

Key words: Hemoglobin, Lead, Reactive Oxygen Species.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing, [2,3]Interpretation and discussion
[4,5] Data analysis, interpretation and manuscript writing, [6] Active participation in data collection.

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Introduction

Lead is a poisonous metal and is widely used in daily life for its good chemical properties. Increased use of lead in industry, its excessive inhalation and ingestion can adversely affect major biological functions in the human body. Heavy metals are among environmental factors that may contribute to (ASD) Autism spectrum disorder.[1] Organic lead, such as tetra-ethyl lead, is a central nervous system toxin that is absorbed through the skin and into the brain; toxicity may occur through handling objects contaminated with organic lead.[2]

Children absorb 40% of a dose of lead compared to 10% in adults and retain 30% of lead absorbed compared to 1% in adults. Blood levels of 10 µg/dl or more in children are proved to be toxic, but even lower levels may impair

development and cognition. Blood lead levels in the general population of developed countries have fallen significantly over the past 20 years due to phasing out of lead petrol and bans on the use of lead in paints.[2-4]

Lead absorption occurs in two forms. Inorganic lead, oxidizes immediately, and can be absorbed through the respiratory tract as lead dust particles and inhalation from lead-containing objects such as paints and lead pipes, through the gastrointestinal tract from water and foods affected by lead contents in the soil, particularly as industrial waste and also from processes such as glazing ceramics, or manufacture of lead accumulators and as a product of lead-containing petrol from atmosphere.[5-7]

In the body 95–99% of ingested lead is sequestered in erythrocytes and dispersed through soft tissues and bone and is also found in hair and nails.[1] Lead after getting absorbed from gastrointestinal tract or lungs enters the blood stream. It attaches to the blood proteins that carry it to various tissues or organ systems in the body.[5] The largest amounts are stored in bone tissue where lead is deposited initially as a colloidal compound and then as a crystalline material. Very low concentrations of lead are found in kidney and liver.[6] In children approximately 65 % is found in skeleton.[7,8]

The elimination of lead from the body is mainly through urine which is 76 % and by gastrointestinal tract is 26 %. About 8% lead is excreted by the sweat, skin exfoliation and hair.[4] Lead is excreted in urine and feces regardless of the route of exposure. The urinary lead excretion rate depends on renal blood flow and glomerular filtration rate. Minor routes of excretion include sweat, saliva, hair, nails, and breast milk.[6-8] Fecal excretion accounts for approximately one-third of total excretion of absorbed lead. The elimination half-life for inorganic lead in blood and bone is approximately 30 days and 20 years, respectively, whereas its half-life in soft tissues accounts for forty days.[9]

Sources of lead exposure and toxicity include old piping, and working in certain occupations such as printing, plumbing, the destruction of old houses, and the use of lead-contaminated vessels in olive oil production, wine flour and canned food. It is also used in ammunition in army, lead dust increases during daily firearm training in an indoor range; it was shown that blood lead levels doubled within the 6-week duration.[10] It is very important to identify the probable sources of lead exposure. Lead based paints were the supreme source of the lead poisoning before 1978, now lead is banned to be used in paint industries in developed countries though it is still being consumed in developing countries. In United State about 74 % of private housing units built before 1980 contain lead based paints.[11-12] Plumbism or Lead toxicity is defined as a toxic condition caused by the ingestion or inhalation of the heavy metal lead. In 1991, the Centers for Disease Control and Prevention of the United States Department of Health and Human Services recommended that lead toxicity occurs when blood lead levels are equal to or greater than 10 µg/dl.[13]

Reproductive toxicology

Lead effects both male and female reproduction adversely. At high concentrations in blood lead acts as a powerful abortificient. At lower levels, it has been associated with miscarriages, and low birth weights of infants. Males may have decreased sperm counts motility, and teratospermia, altered sperm morphology and function.[14,15]

Neurotoxicity

Lead has miscellaneous impacts on the CNS. Immature astrocytes are receptive to lead, and it interferes with myelin formation and of the blood-brain barrier integrity. When the barrier is disrupted, molecular proteins like albumin enter the CNS, resulting in edema, increased intracranial pressure, and encephalopathy.[16] The most commonly predictable adult neurological symptom of lead exposure is peripheral neuropathy.[17] Patients with high blood lead levels may present with severe colic, motor disturbances, altered consciousness, paralysis of limbs and weakness.[18]

Hypertension and cardio vascular affects

Studies have been carried out in animals and humans showing that in animals exposed to lead in drinking water, lead affected the renin–angiotensin system, inducing hyperactivity of sympathetic system and increasing sensitivity to stimulation of cardiac vascular and dopaminergic receptors.[15] High blood lead levels have been associated with high blood pressure.[19,20]

Lead, gout and renal disease

Glycosuria and aminoaciduria (saturnine Gout) are produced from long term exposure of this heavy metal along with damage to the renal tubules. Lead

nephropathy has been well documented in occupationally exposed workers manifesting as interstitial fibrosis, proximal tubular damage and sclerosis of glomeruli.[21]

DNA backbone damage

Backbone damages include single and double-strand DNA breaks. Abasic sites can be generated spontaneously, by the formation of unstable adducts or by base excision repair. SSBs are produced directly by detrimental agents, or the intermediates of base and nucleotide excision repair can give rise to single-strand breaks in the range of 1–30 nucleotide.[22] Along with that when a damaged DNA effort to replicate it brings along with it certain errors or mutations into the genetic code.[23]

Patients and Methods

This case control comparative study was conducted in the Department of Biochemistry, University of Health Sciences Lahore. Study was completed in 12 months after the approval from institutional ethical committee. There are 1600 traffic police wardens in Lahore working in 9 administrative towns namely Ravi town, Shalimar town, Aziz Bhatti town, Wagah town, Data Ganj Bakhsh town, Gulberg town, Allama Iqbal town, Samanabad town and Nishtar town. One hundred sixty traffic wardens work in each town and out of them 100 work in field. Total 60 traffic wardens from nine administrative towns of Lahore, working in morning shift, age range between 25-40 years and having at least 1 year of service in field were selected. They were nominated through stratified random sampling using proportional allocation after taking permission from police central line. Wardens working in closed offices or patrolling in cars, known case of any chronic inflammatory disease such as asthma, known case of any autoimmune disease and smokers were excluded from study. Thirty age and sex matched controls were taken who volunteered for giving blood. Control group was consisted of healthy office workers and students who had never been occupationally exposed to known genotoxic agent. After an informed consent, blood samples were collected from traffic wardens and controls fulfilling the inclusion criteria. All the blood samples were drawn under aseptic conditions from the median cubital vein from anterior aspect of forearm. Total 5 ml blood was obtained. For cell extraction, 2.5 ml of heparinized blood was collected in heparin containing vials and mixed properly and 2.5ml of blood sample was taken in black top metal free nitric acid treated tube. The sample was transported to UHS in a cool box containing ice bags. Cell isolation was performed on the same day of blood collection. Blood lead concentration was determined by atomic absorption spectrometer

Regarding comet assay method for DNA detection, a modified version by Singh and colleagues in 1988, introduced a microgel technique. This involves electrophoresis under alkaline (pH 13) conditions for detecting DNA damage in single cells. The comet assay is a method in which the basic principle is to determine the DNA breaks by measuring the DNA damage which is quantified by the proportion of DNA, which migrates out of the nuclei towards the anode when individual cells or isolated nuclei is subjected to electrophoresis. The image obtained with this technique looks like a comet like shape with an intact head, the nuclear region and a tail which contains DNA fragments of nuclei followed by electrophoresis. The amount of DNA liberated from the head of the comet during electrophoresis depends on the level of effect of the mutagen under evaluation. Images were captured using Charge-coupled device (CCD) camera attached with the microscope (Figure 1).

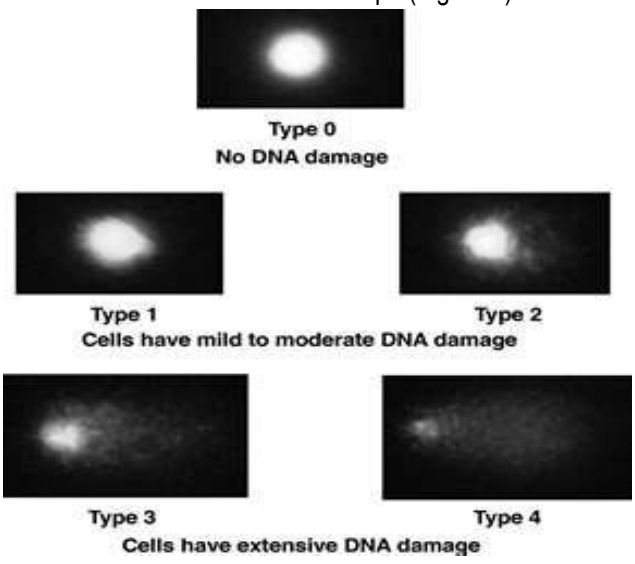

Figure 1. DNA migration pattern produced by the Single Cell Gel/Comet assay. (Carcin.oxfordjournals.org).

The data were entered and analyzed using SPSS version 18.0. For quantitative variables (Lead level, DNA damage, and age), mean ± S.D was given. Distribution of variables was checked using the

Shapiro-Wilk test. Because of non-normal distribution of parameters, the differences between mean values were tested with nonparametric tests the, Mann-Whitney U test. Spearmen correlation was applied to observe correlation between quantitative variables (DNA damage parameters and lead level). Correlation coefficient (r) was determined. p value of ≤ 0.05 was considered as statistically significant.

Results

Out of total 90 participants, 60 were cases and 30 were control. The age range of cases and control was 24-39 years and 25-35 years respectively. Mean age in cases and control was 27.62±2.15 years and 28.13±2.33 years respectively. Mean levels of lead in both cases and control groups were 18.76±8.84 µg/dL and 12.00±3.552 µg/dL respectively. Blood lead levels were significantly increased in cases as compared to controls (Table 1). Variables of DNA damage, tail moment and tail length were significantly increased in cases. Difference of % DNA in tail was non-significant between cases and control (Table 2).

Table 1: Age and blood lead levels of cases and controls (n=90)

Variables	Cases (n=60) median (IQR)	Controls (n=30) median (IQR)	p-value
Age (years)	27.50(26-28)	28.00(27-29)	0.241
Blood lead levels (µg/dL)	17.00(12-22)	11.50(10-13.25)	0.000

Table 2: Variables of DNA damage in cases and controls (n=90)

Variables	Cases (n=60) mean±SD	Controls (n=30) mean±SD	p-value
Tail moment (%)	0.0583±1.960	0.045±0.108	0.04
Tail length (µm)	7.156±12.56	1.50±2.12	0.03
% DNA in tail	4.101±5.87	2.055±2.481	0.136

Difference of other parameters of DNA damage including % DNA in head was non-significant (p=0.16) and comet length was statistically significant between cases and controls (p=0.005). Among cases, lead levels were significantly correlated with tail moment, % DNA in tail and % DNA in head while lead association was non-significant with comet length and tail length (Table 3). Among control, correlation of lead levels was significant with tail moment (r=0.407, p=0.026) while non=significant association was found with comet length (r=0.191, p=0.313), tail length (r=0.226, p=0.231), %DNA in tail (p=0.926) and % DNA in head (p=0.926).

Table 3: Correlation of lead levels with variables of DNA damage in cases (n=60)

Variables	Correlation coefficient	p-value
Tail moment	0.337	0.008
% DNA in tail	0.380	0.03
% DNA in head	-0.380	0.03
Comet length	0.134	0.306
Tail length	0.185	0.15

Discussion

Air pollutants generated from traffic and industrial plants are believed to be one of the major causes of DNA damage in living species. Because of rapid urbanization, air pollution and environmental quality deterioration have been affecting our daily lives as well as the nature. Several experimental studies have reported that lead has a moderate genotoxic potential.

In a study conducted by Valverde and colleagues [24] a lead 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. Following single and subsequent inhalations, differences were found among the organs studied. A positive induction of DNA damage in the liver and the lung after a single inhalation was observed. The response was positive in all organs, except the testicle, in subsequent inhalations. DNA damage induction over time varied for each organ. The brain and bone marrow showed the

highest damage induced. Differences in DNA damage occurred in organs when lead was administered acutely or sub-chronically.

Our study revealed a significant increase in the blood lead level of study group and controls (p value 0.000). The mean pb in our study was 18.76±8.8 and controls mean pb was 12±3.5 as compared to study in Islamabad that was carried out in 2005 in which blood lead levels of wardens were 27.27 ±4.04 and a similar study on traffic wardens in Karachi indicated a value of lead in wardens of 47.7±15.8. micrograms /dl.Another study in Alexandrria Eygpt reported that their traffic constables had a higher blood lead level than our study.[25]

Blood lead levels were analyzed in Nigerian traffic wardens, comprising sixty from Lagos, thirteen from the sparsely populated university town of Ile-Ife and a control group of twenty-four subjects. The mean lead level in Lagos wardens was 18.1 ± 6.4 μg/dl, which was significantly higher than the level of 10.2 ± 2.7 μg/dl in Ife wardens and 12.9 ± 7.0 μg/dl obtained in the controls (P < 0.001). However, there was no significant difference between the levels of blood lead in Ife traffic wardens and normal controls.[26,27]

Nigeria wardens had almost similar values as our study but their study was conducted 17 years ago and since that time urbanization and population has increased tremendously. Alexandria and Karachi both are populated cities and have more traffic congestion that is why their lead levels were high as compared to our study. Also after 2005 when the law of using unleaded petroleum was introduced no study had been conducted since then on this occupationally exposed group. Our study indicated that law had actually been implemented that has reduced lead from the atmosphere and from this exposed group.

The mean age in cases in our study was from 24-39 years and in controls was 25-35 years. There was no statistical difference in the mean age of cases and controls in our study (p value 0.241). The mean age of wardens in the study of Islamabad and Karachi had a mean value of 21-45 years and 20-52 years respectively, not comparable with our study. The various parameters of comet assay that is Mean tail moment, % DNA in tail and Mean tail length in our study had values of 0.0583 ±1.960 (p valve 0.04),4.101 ±5.87 (p value 0.136) and7.156 ±12.56 (p value 0.003) respectively in cases and 0.045±0.108 ,2.055 ±2.481 and 1.50±2.12 respectively in controls. There was a significant correlation of lead with TM, but no statistically significant correlation of lead with tail length in cases. The mean % DNA in tail in cases gave a mild positive correlation with lead.

The other parameters given in our study were % DNA in head with a difference in the mean value of cases and controls with a (p value 0.16). The % DNA in head gave a mild negative correlation with lead in cases (p value 0.03 and rho -0.380). Comet length in our study had a difference in the means of two groups having a p value 0.005 that was statistically significant but it gave no significant correlation with lead in cases. The homeopathic preparation Plumbum metallicum had no effect, in a study conducted by Riera in 2011, in terms of reducing serum lead in workers exposed to lead.[28]

A study of DNA damage by comet assay in Traffic wardens in China [29] showed that occupational exposure to traffic exhaust significantly increased tail length 4.20µm, (3.98 - 4.42) µm in outside ones vs. 3.23 µm, (2.82 - 3.7) µm in office work policemen, P < 0.001. The p value of mean tail length is 0.003 in our cases and controls that is statistically significant and comparable to their study however our study did not give a significant correlation of lead with tail length in cases. The results confirm that inhaling lead induces systemic DNA damage, but certain organs, such as the lung and the liver, are special targets of this metal, partly depending on the duration of exposure.

Conclusion

Because of industrial development, individuals exposed to industrial areas or lead due to their professional work, had significantly higher mean values of blood lead. The correlation coefficient of pb with comet length in cases was statistically insignificant and rho of lead with percentage of DNA in head of cases was mild negative correlation and rho of lead with percentage of DNA in tail of cases was positive correlation and p value in controls was not statistically significant.

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