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Effects of Dust Pollution in Asaluyeh on Atomic Absorption of Heavy Metals and Histopathologic Changes in Spleen and Bone Marrow of Male Rats

Vahid Keshavarz[1], Saeed Rezaee Zarchi[1], Afshar Bargahi[2], Hossein Khorramde[3], Mohammad Ali Zare[4], Zeinab Mahdian[4], Arman Hashemi[5], Mohammad Hashemi[5], Parviz Farzadinia[4*]

Abstract

Objectives: The effects of air pollution from industrial advances and mechanization on people, specifically children whose immune and respiratory systems are not fully developed, have attracted growing attention from researchers. Given that the oil and gas facilities in Asaluyeh have turned it into one of the most polluted regions in the world, this study aimed at investigating the effect of airborne dust particles (ADPs) on the atomic absorption of heavy metals and histopathologic changes in spleen and bone marrow of male rats.

Materials and Methods: In this study, 30 adult male rats were assigned to the control, negative control, and treatment groups. After the course of treatment, the changes in spleen and bone marrow tissue, as well as atomic absorption of metals (lead, mercury, cadmium, and arsenic) in their serums were examined.

Results: The atomic absorption of metals in the serum of the treatment group significantly increased as compared to the control group; in addition, the significant histopathologic changes were observed only in the spleen tissues.

Conclusions: Dust of polluted air of Asaluyeh had relative toxic effects on spleen tissue and serum but did not have toxic effects on bone marrow.

Keywords: Dust pollution, Asaluyeh, Spleen, Bone marrow, Rat

Introduction

Air pollution is one of the greatest threats to human health worldwide, which causes health problems in the long and short run (1). The severity of these problems varies in different groups. For example, the elderly and children suffer more from air pollution (2). Today, nanoparticle pollutants are regarded as a new hazardous issue (3). The harmful effects of dust pollution are mainly associated with the concentration of particles <100 nm, not depending on the mass concentration of larger particles. The probable risks from airborne nanoparticles, i.e. aerosols, are even more serious. This is because of their intense motional activities and a greater chance of being absorbed by the lungs, as the easiest route to enter the body (4). All types of dust can bring about undesirable effects on the cardiovascular system through their different biological mechanisms. The airborne dust particles (ADPs) in the polluted air may cause coagulation disorders, increased risk of thrombosis, endothelial vascular disorders, and arterial contraction through releasing inflammatory substances in the lungs (5,6). On the other hand, increased heart rate and hypertension, as well as reduced natural heart rate provide the context for coronary artery stenosis, heart attack, and heart arrhythmias (7,8). Those who are chronically exposed to air pollution for a long time are more prone to coronary artery stenosis, due to the persistent inflammatory role of ADPs (9). The microscopic airborne particles affect the cardiovascular system and narrow carotids, which may finally result in heart diseases or early death in polluted regions. It has been proved that some pollutants have inflammatory impacts on the artery walls (10). Organic compounds and ADPs have hazardous impacts on humans, animals, plants and ecosystems. Accordingly, the highly polluted air causes serious problems for humans, such as burning eye, nose, and throat, respiratory problems, chest pains, and increased risk of heart attack (11). In many cases, this problem causes cancer, damages immune, neural, and reproduction systems, and even leads to death. Although air pollution evolves in a process from emission from the pollution source, transfer, deformation, and finally elimination from the atmosphere, these changes still affect humans, ecosystems and so on. Since a process for complete elimination of pollution and its effects on ecosystems and

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[1]Department of Biology, Payame Noor University, Taft Center, Taft, Iran. [2]Marine Biotechnology Research Center, Persian Gulf Biomedical Research Center, Bushehr, Iran. [3]Department of Biology, Faculty of Basic Sciences, Kazerun branch, Islamic Azad University, Kazerun, Iran. [4]Department of Anatomical Sciences, Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran. [5]Student Research Committee, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
***Corresponding Author:** Parviz Farzadinia, Tel: +989177723543, Email: bazzy_par@yahoo.com

animals seems impossible to design (12), researchers look for a way to minimize the effect of pollution and even prevent it. This is because ADPs in Iran, specifically in the southern part, are due to the extent of deserts, drought conditions in the Middle East, and environmental factors, such as wind from the Persian Gulf and its oil fields (13,14). Due to its oil and gas facilities, Asaluyeh which is located in the southern part of Iran is among the most polluted regions in the world. Nevertheless, there is no comprehensive study into the effects of regional pollution on the immune system. As a result, we aimed to develop a model capable of measuring the effect of this type of pollution on different parameters in an animal laboratory. To this end, this study qualitatively investigated the effect of this phenomenon on morphological changes and atomic absorption of metals.

Materials and Methods

Selection Mechanism and Keeping Conditions of Laboratory Animals

In this study, 30 adult male Sprague-Dawley rats (approximately 100-120 days old and weighing 150-200 g) were purchased from the Center for Breeding and Raising Laboratory Animals of Bushehr University of Medical Sciences. They were maintained under a 12-hour light/12-hour dark cycle at a constant temperature of 18±2°C. In addition, there was no food or water restriction throughout the experiments.

Classification

In this study, 21 rats were assigned to 3 equal-sized groups:

- Group 1 (dustless): In this group, rats were placed in a dustless environment for 3 weeks.
- Group 2 (non-polluted dust particles): In this group, the rats were maintained in an environment with non-polluted dust particles collected from Gorgan, an area without oil pollutants, for 21 days, 8-hour per day (8:00 to 12:00 and 16:00 to 20:00). The experimental environment was a Dust Air Maker B-110 (a glass aquarium equipped with a fan to circulate the dust flow in the air), fabricated by the Bushehr University of Medical Sciences.
- Group 3 (dust particles containing oil and industrial pollutants): In this group, the rats were maintained in a similar device, containing aromatic hydrocarbon-polluted dust particles, collected from Asaluyeh, for 21 days, 8 hours per day (8:00 to 12:00 and 16:00 to 20:00).

Measurement of Size and Concentration of Dust Particles

A dust sample was transferred to the Applied Meteorology Research Institute of Bushehr, the capital city of Bushehr province, to match the pollution level of the aquarium with actual pollution level in Asaluyeh. The Particle Counter PC200 (Trotech, Italy) was used to measure the size and concentration of dust particles. In terms of the analysis degree, particles had different types.

Blood and Biopsy Sampling

At the end of the treatment, the rats were anesthetized through intraperitoneal injection of ketamine (50 mg/kg) and mesalazine (Pars Azmoon Company) with a ratio of 1:3. Then, the blood and biopsy samples were taken.

After anesthesia, blood sampling was carried out directly from the heart. Approximately, 5 mm of the blood of each rat was collected. The blood samples were gently collected in test tubes and placed at room temperature to clot. They were then centrifuged at 3000 rounds per minute for 5 minutes to obtain the serum. In the next stage, the serums were collected in separate vials (1.5 mL), using a sampler, to perform atomic absorption, white cell counting, and CRP tests. The blood serums were then placed at -20°C and transferred to the laboratory the same day by holding the cold chain.

After collecting the blood samples, the spleen sample and bone marrow smear (0.5 mL) from the femoral head were collected, rinsed in physiological serum, and placed in formalin 10%. Then, other preparation stages including draining, saturating, molding, and cutting (sections of 5 microns), and finally staining through standard and routine procedures were carried out in the Histopathology Laboratory of Bushehr University of Medical Sciences. The preparation and staining of the blood smear (Giemsa staining) were performed in the Hematology Laboratory.

Atomic Absorption of Heavy Metals

The atomic absorption device is used to measure the metal content. The samples introduced to the device should be in form of solutions.

CRP Methodology

The markers and samples were subjected to the room temperature.

- One drop of serum, positive control, and negative control was placed in separate test circles of the slide. The serum samples were diluted (1:20) with kit buffer (50 mL).
- The sensitized latex particles were homogenized by slight shakes, and then one drop of it was added to each test circles.
- The solutions were shaken using a rotator and rotational hand movements for 2 minutes and observed for agglutination.

White Cell Counting

After blood sampling and heparinization, the samples were taken to the hematology unit and leukocytes were counted.

Data Analysis (Statistical Methods)

In this study, data analysis was carried out in SPSS version 17.0. In addition, diagrams were drawn in Excel.

The descriptive statistics for quantitative variables were presented in the form of mean and standard deviation. The normal distribution of data was confirmed using the Kolmogorov-Smirnov test. To compare the mean of quantitative variables between the experimental and the control groups, the independent *t* test was used. In addition, the one-way ANOVA was employed to make between-group comparisons. The Pearson correlation was used to investigate the relationship between quantitative variables. In this study, $P < 0.05$ was considered to be statistically significant.

Results

Results From Measurement of Atomic Absorption Parameters

Table 1 presents the results of the comparison of the health level and tolerability to the uptake of toxic heavy metal (lead, cadmium, mercury, and arsenic) in all groups. According to statistical analyses, the amount of toxic heavy metals (lead, cadmium, mercury, and arsenic) was higher in Group III than the other two groups. However, the changes in other groups were not significant.

Tissue Examination Results

Results From Bone Marrow Tissue Examinations in Different Groups

According to Figure 1, bone marrow tissues, such as bone blades (white arrow) and hematopoietic colonies (black arrow) have quite uniform, regular, and healthy strength; there is no histopathologic change, and cell concentration is normal in all groups.

Results From Spleen Tissue Examination in Different Groups

Figure 2 shows the cross-sectional optical photomicrograph of bone marrow in different groups at the end of the treatment. According to this figure, all spleen tissues in group I, including the white pulp (white arrow) and red pulp (white arrow), have uniform, regular, and normal strength, there is no specific histopathologic change, and cell concentration is normal; whereas, these tissues in other groups, specifically group III, had a quite irregular, nonuniform, and normal strength, specific histopathologic changes, such as hyperemia, necrosis, atrophy and abnormal dilatation in the red pulp, and unnatural cell concentration.

Discussion

Results From Measurement of Atomic Absorption Parameters

Heavy metals in ecosystems are a potential risk for living animals. Humans are always subjected to heavy metal pollution. These metals bond with essential body components, such as oxygen, sulfur, and nitrogen. The majority of essential body components, including enzymes and proteins, are in this group. As a result, heavy metals interrupt enzymatic activities and disrupt the synthesis of essential body compounds. The main issue associated with heavy metals is that they are not metabolized in

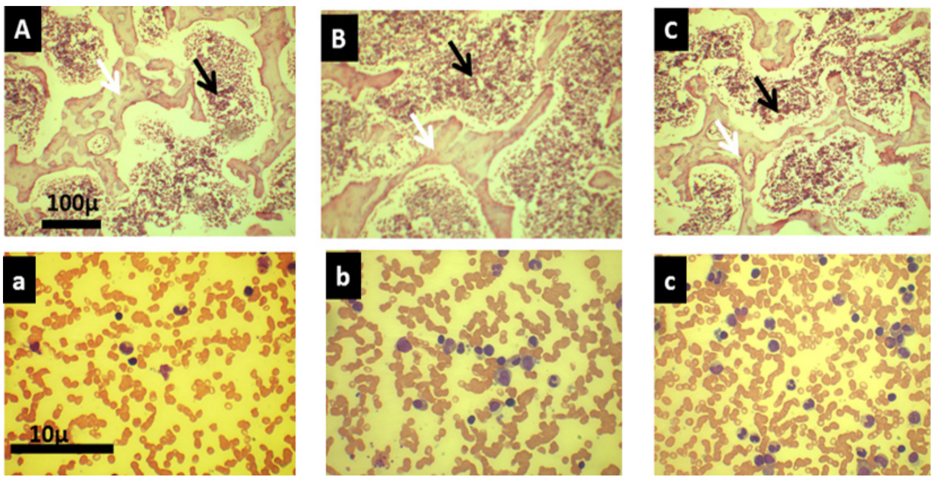

Figure 1. Cross-Sectional Optical Photomicrograph of Bone Marrow in Different Groups at the End of the Treatment; Group I (A, a), Group II (B, b), Group III (c, c), bone blades (white arrow), and hematopoietic colonies (black arrow), magnification 100X (top images), magnification 400X (bottom images), normal staining

Table 1. Atomic Absorption in Different Groups

Group I	Standard	Group I	Group II	Group 3
Lead (ppm)	0.012±0.005	0.013±0.001	0.015±0.010	0.092±0.007[a,b]
Cadmium (ppm)	0.0049 ± 0.0010	0.0052 ± 0.0005	0.0063 ± 0.009	0.0227± 0.001[a,b]
Arsenic (ppm)	0.0058±0.0007	0.0068±0.0001	0.0072±0.0010	0.0260±0.0013 [a,b]

Group I, clear air as control group; group II, non-oil polluted air; group III, oil polluted air collected from Asaloyeh, Iran. Data was analyzed using t-test and f-test (n=10, Mean±SD, $P < 0.05$). [a] Significant difference with control group; [b] Significant difference with group II.

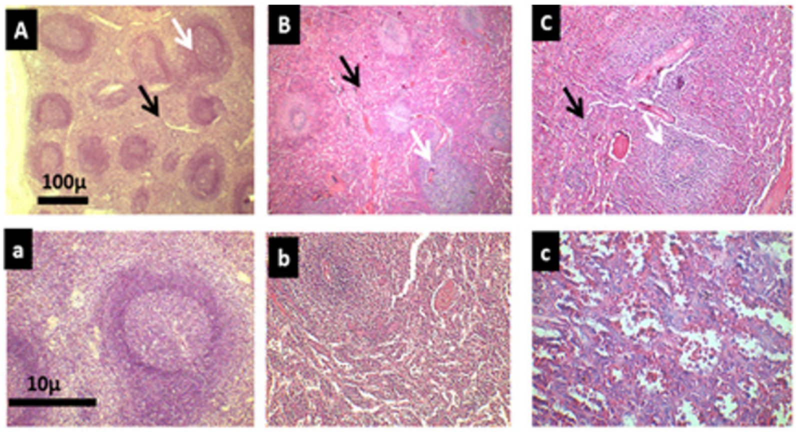

Figure 2. Cross-sectional Optical Photomicrograph of Spleen Tissues Bone Marrow in Different Groups at the End of the Treatment; Group I (A, a), Group II (B, b), Group III (C, c), Magnification 100x (top images), magnification 400x (bottom images), normal staining.

the body. In fact, since the body cannot dispose of heavy metals, they accumulate in tissues of liver, spleen, muscles, bones, and joints, resulting in the development of many diseases and complications.

Results showed a significant increase in all toxic metals (lead, cadmium, mercury, and arsenic) in group III as compared to the other two groups. In addition, the changes in the other groups are not significant.

It is worth noting that ADPs in Iran, specifically in the southern part, are because of (the extent of) deserts, drought conditions in the Middle East, and environmental factors, such as wind in the Persian Gulf and its oil fields. Due to its oil and gas facilities, Asaluyeh which is located in the southern part of Iran is among the most polluted regions in the world. The high level of airborne heavy metals can be attributed to the multiplicity of oil facilities, oil wells, and extraction of large volumes of petroleum products. The entry of these substances into the blood through respiration may have biological effects on the body.

Investigating and Discussing Tissue Examination Results

In the present study, the histopathologic and morphological changes of the tissues of the immune system of adult male rats were examined by in-vitro exposure to air pollution. According to histopathologic and morphological findings, exposure to such pollutants can induce changes in the tissues of the immune system, specifically in spleen, which is a highly congested tissue. These changes included morphological and morphometric changes. The morphological changes include changes in the tissues of the immune system, such as intercellular impairment in the interstitial tissues, which have an irregular and nonuniform strength, indicating histopathologic changes. According to epidemiological studies conducted in 2017, those groups, infants and children in particular, who were exposed to air pollution, specifically heavy metals (e.g. lead), for a longer time were more prone to cardiovascular and immune system problems. In general, the findings of the present study were consistent with those of other studies. Moreover, its toxic effects on the immune system were greater in highly exposed body parts such as kidney, myocardium, and neural and immune system (15,16). Since tissue homeostasis depends on the relationship and balance between cellular reproduction, differentiation, and death, a balance between these processes can result in natural tissue function; whereas, lack of balance between them can lead to anatomic and physiological impairments of the immune system (15,16). According to the results, the hazardous changes were greater in group III than in the other groups (17).

Since the origin of initial changes in each tissue is cellular damage, which is caused by damaged DNA, the mechanism of extensive histopathologic changes should be addressed first.

DNA Oxidative Degradation and Damage Mechanism

The chemical reaction of DNA to endogenous chemical materials and radiation can change chemical and physical structures of DNA. These changes can inhibit transcription and may be fatal. In other words, they can cause a physical DNA deformation, which may lead to transcription inhibition or cellular death. Under natural conditions, the oxidative degradation occurs in all aerobic cells due to the presence of reactive oxygen species, such as hydrogen superoxide and in particular hydroxyl radicals. These radicals can attack DNA in some points and produce a range of oxidative products, such as 8-oxoguanine, 2-oxyadenine, and 5-formyluracil. The amount of these oxidative substances increases with free hydroxyl radicals (18,19).

Conclusions

According to the above-mentioned studies, since protein molecules form the base structure of enzymes and protein-protein interaction regulates enzymatic activities (e.g. protein kinase enzyme that plays a role in hormonal activity and forms R2C2 tetramer), it can be said that the enzymatic inhibition is among the most hazardous effect of lead on the tissues of living animals (20). Therefore, it is recommended to use less risky metals in industrial, production, and consumption areas, and take extra caution in using lead-containing compounds. It is also recommended to enrich canned foods, specifically those used more by children, with strong antioxidants such as vitamin C. An increase in daily consumption of fresh vegetables, fruits, seeds and so on, which contain antioxidants, is also recommended (21).

Conflict of Interests

Authors have no conflict of interests.

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