Effects of Dust Micro Particles of Asalouyeh Area on the Thyroid and Parathyroid Glands in Rat

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Abstract

Objectives: The aim of this study was to evaluate possible changes in thyroid and parathyroid glands tissues and functional structures.

Materials and Methods: About 30 adult male rats were divided into 3 groups: control, negative control (exposed to dust in the clean area free from oil contaminants), and the treatment group (exposed to dust in the polluted area of Asalouyeh with oil hydrocarbons). Before grouping, all the animals were co-cycled. After 25 days of treatment, changes in pancreatic tissue and serum levels of thyroid and parathyroid hormones were measured. Moreover, the amount of heavy metals including lead, cadmium, arsenic, and mercury in animals’ serum were measured using atomic absorption.

Results: The amount of heavy metals like lead, cadmium, arsenic, and mercury showed a significant increase in the serum of animals in treatment group compared to negative control and also control groups ($P \leq 0.05$). Serum levels of thyroid and parathyroid hormones in treatment group showed a significantly decrease in comparison with control groups. In addition, histological investigations demonstrated relative changes in tissue and functional structures of important thyroid and parathyroid glands tissues.

Conclusions: Dust of Asalouyeh polluted air had relative toxicity effects on thyroid and parathyroid tissues and their hormones.

Keywords: Dust, Air pollution, Asalouyeh, Thyroid, Parathyroid

Introduction

Metals and metal compounds play an important role in living organisms. However, excessive exposure to heavy metals has toxic effects (1). Heavy metals are persistent and widespread pollutants that affect the structure and function of several organs through production of oxidative stress (2). Several studies have been conducted on toxicity of every environmental heavy metal in living organisms (3) and in all of them, the harmful effects of heavy metals on human health have been approved (4). Heavy metals lead to oxidative damage through different mechanisms. Reactive oxygen species (ROS) cause injury and cell death as well. Overproduction of ROS can cause changes in sub-cell structures. These changes include modifications in the structure of proteins and DNA, lipid peroxidation of unsaturated fatty acids, and cell antioxidant system. Cells can send antioxidant and detoxification responses to the effects of heavy metals (5). Antioxidant enzymes such as glutathione peroxidase, glutathione-s-transferase, superoxide dismutase, and catalase play an effective role in protecting cells from oxidative stress (6). Many previous studies have shown that heavy metals can cause histopathological changes in many tissues (7). Small amounts of many metals such as copper, magnesium, sodium, potassium, calcium, and iron have an essential role in the proper functioning of biological systems (8). However, metals, at higher doses, can have toxic effects; exposure to high levels of environmental metals can also cause diseases in humans (9). Nanoparticles made of these metals have some effects on human health. Previous studies on inhaling those nanoparticle particles in non-nanoparticulate sizes or laboratory research on healthy animals have shown that nanoparticles smaller than 30 nm enter the blood circulation system very fast while non-metallic nanoparticles with sizes between 4 and 200 nm rarely enter the bloodstream or do not enter blood at all (10). Instead, people who suffer from respiratory and cardiovascular diseases have greater capillary permeability...
and non-metallic and metallic nanoparticles can quickly enter into their bloodstream (11). Little is known about the potential effects of nanoparticles on human health, especially their toxic effects on endocrine glands system. This is very important because endocrine glands dysfunction is connected with severe adverse effects on human health. Moreover, considering the existence of heavy metals such as zinc, copper, iron, and nickel in soil and dust of Asalouyeh public area as well as oil and gas installations, this region is considered as one of the most polluted regions of the world. Heavy elements are the ones that have atomic mass more than iron, which are greatly absorbed to living tissues, deposit there, and hardly exit the tissue; they include zinc, cadmium, cobalt, copper, lead, nickel, arsenic, vanadium, and chromium (12). Examining the amounts of iron, copper, zinc, and nickel in dust of Asalouyeh it was found that iron and copper had the highest and lowest values in the dust and soil of this area among the four elements, respectively. In the past 50 years, epidemiologic data have indicated high prevalence of human health problems including disturbance in the process of fetus development, disorders of the nervous and immune systems, reduced fertility, diabetes, obesity, and also breast, ovarian, testicles, and prostate cancers. (13). The justification for these observations was that increasing growth of the workers and general population exposed to pollutants could make these pollutants act as endocrine-disrupting chemicals (EDCs). In fact, most research conducted on EDCs has revealed that these compounds can cause the above-mentioned diseases through altering the hormonal system and homeostasis. The harmful effects of particulate air pollution are mainly related to the concentration of particles smaller than 100 nm and are not much dependent on mass concentration of large particles. The risks of nanoparticles in the air i.e. aerosols are of great importance. This becomes important due to their high mobility and possibility of their absorption through the lungs that is the most convenient way to enter the body (14). The results of the study by Casals-Casas and Desvergne showed that nanoparticles were capable of exerting cytotoxic effects and destroying the cells forming endocrine glands leading to disruption in endocrine glands system. Consequently, it was found that they affect endocrine homeostasis, biosynthesis, and releasing of hormones, change the metabolism, and interfere with ligand messaging path (15).

So far, no comprehensive research has been carried out on the pollution effects of this region on rats. Moreover, considering the existence of heavy metals like zinc, copper, iron, and nickel in soil and dust of the public area of Asalouyeh and that the existence of oil and gas facilities installations making the region one of the most polluted regions in the world, in this study, it was attempted to evaluate the effects of dust in the polluted area of Asalouyeh on tissue changes in liver and kidney and also serum factors indicating liver damage in the rats.

### Materials and Methods

#### Statistical Population and Sample

Forty-five male Sprague-Dawley rats within the age range of 120-140 days and weighs of approximately 180-220 g were purchased from laboratory animals breeding center of Tehran Pasteur Institute (IPI).

#### Keeping Conditions

All of the rats were kept in cages under controlled temperature conditions (24 ± 3°C), that is, humidity of 55% to 65%, and a 12-12 light-dark cycle for 2 weeks under free regimen in order to cope with the conditions and get used to the new environment. Special compact foods (Pellets) were produced by Livestock and Poultry Feed Company (LPF, Iran). Water and food were provided for the animals during the entire treatment period without any restrictions. Working with laboratory animals in all the stages of research was in accordance with the standards and regulations of “ethical use of laboratory animals” codified by Mobasher et al.

#### Grouping

Animal were randomly divided into 3 equal groups (each group consisted of 15 rats) as follows:

1. First group (control) was kept in a dust-free environment (clean room) for 3 weeks.
2. The second group (negative control) was kept (for 21 days, for 8 hours per day — 8-12 pm and 4-8 pm) in an environment contaminated with dust collected from dust-contaminated environments but free of petroleum contaminants in a simulated environment that was designed by a research team in Bushehr University of Medical Sciences (BPUMS.E-415). A system fan inside the glass aquarium constantly circulated dust in the environment where the animals were located.
3. Third group (treatment) was exposed to the dust collected from the polluted area of Asalouyeh, where the largest oil installations of Iran are located (this city is located in south area of Iran); It was contaminated with petroleum aromatic hydrocarbons for the same period of time.

#### Treatments

Animals were treated during a 4-week period (28 days). They (groups I and II) were put inside a special cage and then were transferred into the machine exposed to the dust. These animals were treated for 28 days (8 hours – 4 hours in the morning and 4 hours in the afternoon – per day).

#### Taking Biopsy and Blood Sampling

**Blood Sampling**

At the end of treatment, the animals were removed from the device and kept fasting for 12 hours and then were anesthetized and sacrificed. Their blood samples were
taken directly from the left ventricle. About 3 mL of blood was drawn from each animal. Blood samples were gently poured in test tubes and placed at room temperature for 1 hour to clot. They were then centrifuged at 3000 rpm for 5 minutes to obtain the serum.

Assessment of Atomic Absorption Spectrometry of Heavy Metals

AAS (Varian Company- odelAA240, Australia) was used to measure the serum levels of lead, cadmium, arsenic, and mercury. The device consisted of a radiation source, sample holder, wavelength selector, detector, and also signal and stability processor. The samples were put in special chamber (Atomizercells) of AAS machine.

Hormonal Assessments

Hormonal measurements were carried out at Razi Laboratory in Bushehr using a model of Cobas e 411 Alexis machine and employing the electrochemiluminescence (ECL) method.

Biopsy, Tissue Processing, and Histopathological Study

Samples (biopsy) were taken at the end of treatment after anesthetizing the rats by a 3:1 mixture of ketamine and xylazine (50 mg/kg I.M). It was performed by sterilized surgical set from declared area of the tissues (thyroid and parathyroid gland). The specimens were fixed in 10% formalin and referred to the histopathology lab. For the light microscopic study, samples were dehydrated and embedded in alcohol (ethanol) and paraffin, respectively. They were cut at thickness of 3 microns by a rotary microtome and stained normally (H & E). Then, the microscopic slide photos were taken by microscope equipped with a Moticam camera model A352 (the Netherland) in a high magnification ion (×100). The obtained results involved the observed changes in histopathological changes such as congestion and tissue damages in different groups.

Statistical Analysis

The collected data was analyzed using SPSS (Statistical Package for the Social Sciences) software, version 17. Charts were drawn using Excel. In addition, descriptive statistics for the studied quantitative variables were presented as mean and standard deviation (SD). The Kolmogorov-Smirnov test confirmed normal distribution of the data. Independent t test was used to compare the mean quantitative factors in each of the test groups with the control groups. Besides, one-way ANOVA was used to compare mean variables between the groups. Pearson correlation test was also used to investigate the correlation between the quantitative variables. The P<0.05 was considered significant in all the analyses.

Results

The Results of Heavy Metals Assessment

Table 1 shows the amounts of heavy metals (lead, arsenic, cadmium, and mercury) which were measured using atomic absorption techniques. As can be seen, the mean of plumb in standard rate, control, and test groups (T1 and T2) was 0.012 ± 0.005, 0.0150 ± 0.002, and 0.32 ± 0.005, and 0.096 ± 0.006, respectively. Data analysis demonstrated that the mean of plumb increased significantly just in T1 and T2 group (Asalouyeh petroleum air pollution) compared to control group (P<0.05). The mean of Cadmium (Cd) in standard rate, control, and test groups (T1 and T2) was 0.0047 ± 0.0009, 0.0053 ± 0.0004, 0.0098 ± 0.0010, and 0.0225 ± 0.001, respectively. Data analysis showed that the mean of Cd increased significantly just in T1 and T2 group compared to control group (P<0.05). The mean of arsenic (Ad) in standard rate, control, and T1 and T2 groups was 0.0058 ± 0.0007, 0.0068 ± 0.001, 0.088 ± 0.0011, and 0.0561± 0.001, respectively. Data analysis revealed that the mean of Ad increased significantly just in T2 group compared to the other groups (P<0.05). The mean of hydrargyrum (Hg) in standard rate, control, and T1 and T2 groups was found to be 0.0009 ± 0.0001, 0.0016 ± 0.0002, 0.0018 ± 0.001, and 0.0047 ± 0.0002, respectively. Data analysis showed that the mean of Hg increased significantly just in T2 group compared to the other groups (P<0.05).

The Results of Hormonal Assessment

Table 2 shows the mean of hormones in control and T1 and T2 groups. As the results show, the mean of parathormon (PTH) in control and T1 and T2 groups increased significantly just in T2 group (Asalouyeh petroleum air pollution) compared to control group (P<0.05). The mean of parathormon increased significantly just in T2 group compared to control group (P<0.05). The mean of parathormon increased significantly just in T2 group compared to control group (P<0.05).

Table 1. Mean of Atomic Absorption Parameters in Different Groups

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Standard Rate</th>
<th>Groups II</th>
<th>Groups III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plumb (Pb) (ppm)</td>
<td>0.012 ± 0.005</td>
<td>0.015 ± 0.002</td>
<td>0.032 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cadmium (Cd) (ppm)</td>
<td>0.0047 ± 0.0009</td>
<td>0.0053 ± 0.0004</td>
<td>0.0098 ± 0.0010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arsenic (Ad) (ppm)</td>
<td>0.0058 ± 0.0007</td>
<td>0.0068 ± 0.0010</td>
<td>0.0088 ± 0.0011</td>
</tr>
<tr>
<td>Hydrargyrum (Hg) (ppm)</td>
<td>0.0009 ± 0.0001</td>
<td>0.0016 ± 0.0002</td>
<td>0.0018 ± 0.0001</td>
</tr>
</tbody>
</table>

Group (II), Clear air as control group; Group (II), non-oil pollutant air; Group III, oil pollutant air from Asaloyeh area, Iran. Data were analyzed using t test and f test methods. (n =10, means = SD, P<0.05).

Note: Significant difference with control group (<sup>a</sup>); Significant difference with group (II) (<sup>b</sup>).
was 3.01 ± 0.65, 2.41 ± 0.89 and 1.86 ± 0.37, respectively. Data analysis indicated that the mean of PTH decreased significantly in T1 and T2 group as compared to control group. Besides, the mean of calcium (Ca) in control and T1 and T2 groups was 10.4 ± 2.46, 9.7 ± 2.12, and 6.2 ± 1.28, respectively. Data analysis showed that the mean of Ca decreased significantly just in T2 group compared to the other groups (P<0.05). Furthermore, the mean of thyroid hormone (TSH) in control and T1 and T2 groups was 3.21 ± 1.03, 1.13 ± 0.88, and 0.31 ± 0.01, respectively. Data analysis denoted that the mean of TSH decreased significantly in T1 and T2 group as compared to control group (P<0.05). The mean of T3 in control and T1 and T2 groups was 2.41 ± 0.06, 1.30 ± 0.16, and 0.21 ± 0.02, respectively. Data analysis revealed that the mean of T3 decreased significantly in T1 and T2 group compared to control group (P<0.05). In addition, the mean of T4 in control and T1 and T2 groups was 8.03 ± 2.06, 6.13 ± 29.28, and 2.71 ± 8.19, respectively. Data analysis showed that the mean of T4 decreased significantly in T1 and T2 group compared to control group (P<0.05). Moreover, the means of stress hormones such as cortisol and ACTH (adrenocorticotropic hormone) were evaluated. As the outcomes show, the mean of Cortisol in control and T1 and T2 groups was 185.23 ± 29.11, 169.42 ± 26.41, and 121.16 ± 32.09, respectively. Data analysis indicated that the mean of Cortisol decreased significantly just in T2 group compared to the other groups (P<0.05). Besides, the mean of ACTH in control and test T1 and T2 groups was 29.33 ± 10.13, 25.12 ± 19.20, and 19.02 ± 11.10, respectively. Data analysis showed that the mean of ACTH decreased significantly just in T2 group compared to the other groups (P<0.05).

Histopathological Findings

Figure 1 shows the light micrographs sections of thyroid and parathyroid gland tissues in control and T1 and T2 groups. As can be seen, in T2 group compared to other groups, the rats lost their regular arrays and many abnormal spaces with congestion were seen between the cells. Meanwhile, the cells of glandular epithelium highly lost their cellular arrays; cellular nucleus were highly heterochromatic and pyknotic; some lymphatic infiltrations were observed in T2 group compared to the other groups.

In addition, a lot of pathological changes such as congestion, irregularity, nuclear density, dispersion tissue were seen in thyroid and parathyroid gland tissues in T2 group compared to the other groups (Figure 1).

Conclusions

Serum levels of TSH (T3 & T4) in all the study groups decreased. In addition, relatively irregular and uneven consistencies and changes in histopathology including congestion, atrophy, and abnormal dilatation in follicles and interstitial tissues were observed in the treatment and negative control groups. Moreover, examining the values of lead, cadmium, arsenic, and mercury in the serum of animals using atomic absorption showed a significant increase of these elements in the treatment group compared with the control group (P<0.05). Although exposure of the thyroid gland to EDCs could have an effective role in overall health, research conducted on most chemicals regarding their potential for disrupting the thyroid was often diverged and had no reasonable and solid results (17). Serum levels of cadmium and lead in the negative control group showed a significant increase compared to pristine control group. Thus, due to the devastating effect of these elements found in previous research, the role of these 2 elements in reducing TSH and pathological tissue changes in thyroid could be expected in the present study as well. However, serum increase of mercury and arsenic in the groups under investigation (along with the increase in lead and cadmium) could be involved in increasing the thyroid destruction and further reduction of TSHs in animals in the treatment group.

Cadmium is among the heavy metals found in abundance in environmental matrices. As has been pointed out in

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<th>Groups II</th>
<th>Groups III</th>
</tr>
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<tbody>
<tr>
<td>Parathormon (mmol/L)</td>
<td>3.01 ± 0.65</td>
<td>2.41 ± 0.89</td>
<td>1.86 ± 0.37ab</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.4 ± 2.46</td>
<td>9.7 ± 2.12</td>
<td>6.2 ± 1.28ab</td>
</tr>
<tr>
<td>TSH</td>
<td>3.21 ± 1.03</td>
<td>1.13 ± 0.88a</td>
<td>0.31 ± 0.01ab</td>
</tr>
<tr>
<td>T3 (ng/dL)</td>
<td>2.41 ± 0.06</td>
<td>1.30 ± 0.16</td>
<td>0.21 ± 0.02ab</td>
</tr>
<tr>
<td>T4 (ng/dL)</td>
<td>8.03 ± 2.06</td>
<td>6.13 ± 29.28a</td>
<td>2.71 ± 8.19b</td>
</tr>
<tr>
<td>Cortisol</td>
<td>185.23 ± 29.11</td>
<td>169.42 ± 26.41</td>
<td>121.16 ± 32.09</td>
</tr>
<tr>
<td>ACTH</td>
<td>29.33 ± 10.13</td>
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Group (I), Clear air as control group; Group (II), non-oil pollutant air; Group III, oil pollutant air from Asaloyeh area, Iran. Data were analyzed using t test and f test methods. (n =10, mean± SD, P<0.05).

Note: Significant difference with control group (a); Significant difference with group (II) (b).
previous research, the values of this metal in soil and water resources depends on the release of heavy metals from various pollution sources (18). Various researchers have confirmed the effects of heavy metals on hormones. In one study, plasma values of gonadotropin, prolactin, ACTH, growth hormone (GH), and TSH were measured in adult male Sprague-Dawley rats that had received cadmium chloride (CdCl2) in their drinking water at doses of 5, 10, 25, 50, or 100 ppm for 1 month. The results showed that cadmium distinctively affected secretion patterns of pituitary hormones such as gonadotropin, prolactin, ACTH, GH, and TSH. Observations made in various studies revealed that cadmium destroyed the structure and function of follicular and para-follicular cells and that its severity increased with an increase in exposure to more cadmium (19).

According to previous studies, lead toxicity can affect different routes of homeostasis of Kelsey Thorpe hormones (calcium-stimulant) and bone metabolism. Lead exists in mineral matrix of bone that contains 90% of the body load. It causes disruption of enzyme activity, inhibition of the absorption of minerals, inhibition of protein synthesis (it can connect to protein sulphydryl agent), reduced availability, and reduced sulphydryl (sulfur) antioxidant reserves in the body. Although kidney is a major organ exposed to toxicity of lead salts, calcium homeostasis in mice are exposed to lead changes (20,21). In addition, lead reserves of bone and their release from the bone leads to general physiology of kinetics of bone calcium. As a result, lead can interfere with bone metabolism and calcium. Calcium and lead in bone are regulated through homeostasis of bone remodeling that is controlled by Kelsey Thorpe, hormones, and other cytokines (22). In addition, this metal can inhibit synthesis of 1,25-dihydroxyvitamin D3 (Kelsey Troll) by affecting 1 alpha-hydroxylation of vitamin D (25-hydroxyvitamin D). Thus, it can compete with calcium, inhibit the absorption of calcium from the digestive tract, and in conjunction with low doses of Kelsey Troll induce hypocalcaemia.

Research has shown that lead increases or decreases the amount of Kelsey Troll and calcium absorption in laboratory animals. The research conducted by Hinther et al on rats showed that an increase in exposure to lead was associated with the reduction in bone development and its density, especially in the period before birth (23). In this study, distinctive histopathological changes of parathyroid including congestion, atrophy, and abnormal dilatation in follicle and interstitial tissues were observed in treatment and negative control groups. Therefore, it seems that parathyroid tissue damage had been due to the effects of dust in polluted area of Asalouyeh in the study group, so that it had caused a reduction in synthesis and secretion of parathyroid hormone and ultimately reduced calcium. If dusts at the level of metabolism of calcium and without affecting parathyroid glands had caused blood calcium, parathyroid hormone secretion should have increased, but in this study, the secretion of this hormone decreased showing the impact of dust on the surface of parathyroid tissue. Mercury (Hg) is a metal widely used in the foundry industry, mining, and manufacturing industries; besides, it is a part of electrical equipment and medical products such as thermostats, thermometers, dental amalgam, switches, and batteries (24). The harmful effects of mercury on cells producing steroid hormones were confirmed by administration of 10 to 100 M mg HgCl2 to Leydig cells, adrenal in the testes, and adrenal of Sprague-Dawley rats. Treatment of animals with mercury reduced corticosterone and testosterone production (25).

**Conflict of Interests**
Authors have no conflict of interests.

**Ethical Issues**
Ethical Committee of Bushehr University of Medical Sciences approved the study (ethics No. 687.25. Date 2016.2.12).
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