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Original Research

Oxidative Stress, Erythropoietin, and Some Physiological Parameters of Workers Exposed to Heavy Metals Pollution

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Abstract

Environmental air pollution directly affects human health, increasing the prevalence of mortality that results from cardiovascular and metabolic disorders. The present study aimed to investigate the effect of air pollution results from the Erbil steel factory on oxidative stress markers, blood erythropoietin hormone (EPO) concentration, cardiovascular and respiratory systems. The study included 250 male samples (150 workers worked in different locations in the Erbil steel factory and 100 healthy males were used as a control group which was selected in the rural area away from the steel factory). A significantly higher concentration of the oxidative marker malondialdehyde MDA and EPO hormone was observed in the serum of workers who work near the furnace part of the factory when compared with control, manager, and scrap parts of the factory. The higher concentration of the MDA and EPO hormone was found in the groups of more than 11-15 years working when compared with the groups of less than 6-10 years working. The results found a significant positive correlation between Pb ($r = 0.85<0.01$), Co ($r = 0.70<0.01$), Hg ($r = 0.74<0.01$) and As ($r = 0.60<0.05$) with MDA. The workers in different locations parts of the factory showed significantly lower values of red blood cells (RBC), hemoglobin (Hb), hematocrit, and forced vital capacity (FVC) and higher red cell distribution width (RDW), systolic blood pressure (SBP), and heart rate as compared with the control group. The decrease and increase of the above-mentioned studies' parameters are time-dependent and differ according to the years of working.

Keywords: oxidative stress, pollution, heavy metals, Steel factory, cardiovascular system

Introduction

In critical need of attention is heavy metal contamination, especially in rapidly industrializing countries [1]. Anthropogenic activities such as the extraction of natural resources, metal smelting, chemical manufacture, industry discharge, and sewage irrigation are the main sources of heavy metal contamination [2].

Following its formation in the Erbil Kurdistan region in 2006, Erbil Steel Company (ESC) began integrated steel manufacturing in December 2006. Currently, the ESC is the most significant heavy industry investment

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in the area, producing an average of 700 tons of scrap iron per day, which is used as the primary raw material for the production of steel bars. The majority of the created or melted material in the furnace, which melts all incoming raw materials at 1700ºC, is made up of heavy metals like cadmium, chromium, lead, and nickel. Additionally, ESC has a fume extraction system, which pollutes the environment. The factory's surrounding area will have a higher concentration of heavy metals due to the ESC's impact on soil characteristics [3]. The results of [4] indicate that the soil samples from the Erbil Steel Factory included more metals than other soil samples (apart from Al). The findings of the metal occurrence analysis showed that Cd was the least prevalent metal, whereas Fe was the most dominant.

Worldwide, environmental air pollution is a significant risk factor for morbidity and mortality. Environmental air pollution directly affects human health, increasing the prevalence of mortality that results from lung diseases, cardiovascular and metabolic disorders [5]. Continuous exposure to metals creates a threat to mankind because they are permanent and non-biodegradable [6]. According to earlier research, employees are generally more likely to be exposed to these metals, which represent 0.2% of the overall health burden. Occupational exposure affects between 0.5 million and 1.5 million people annually in developing nations. [7].

Oxidative stress is one way that heavy metals can harm a person's health. Reactive oxygen species (ROS) can be produced and overproduced by several heavy metals due to their oxidation-reduction (redox) capabilities when antioxidant defenses are insufficient to stop this from happening. As a result, excessive ROS generation causes oxidative damage [8]. Recent investigations have revealed a link between increased oxidative stress and exposure to heavy metals. People who live in high-exposure areas have regularly been reported to have higher levels of oxidative stress due to industrial exposure to heavy metals [9].

Heart and blood vessel illnesses are collectively referred to as cardiovascular diseases (CVDs) [10]. In 2016, 31% of deaths worldwide were attributed to CVDs, and heart attacks and strokes account for 85% of all CVD deaths [11]. Air pollution, tobacco smoke, toxins, and heavy metals are only a few environmental risk factors that have been linked to increased cardiovascular system vulnerability [12]. Environmental toxic heavy metals play a significant role in cardiovascular risk, according to a recent systematic review and meta-analysis by [13]. Other diseases coincide with air pollution such as Metal fume fever, asthma, and emphysema [14].

The heavy metals are deposited as aerosols, dust, and steam in the bronchioles and alveoli of the airways and severely harm the lungs [15]. Heavy metals can enter the blood circulation system through particles and create systemic inflammation, which may exacerbate lung damage [16]. In addition, other routes including food and skin may also contribute to exposure to heavy metals, which may be directly or indirectly associated with lung health [17, 18].

Unaware of their effects on environmental health, many industrial units have been built in the previous 20 years, including a steel factory in the Erbil Governorate. This factory released or expelled a significant amount of particular matters and waste into the neighborhood, which is hazardous to both human and animal health. Since there is no information and data regarding biological surveillance or the impact of heavy metals on human health, particularly that of industrial workers. So, the present study was done and aimed to detect the oxidative stress marker, serum erythropoietin, and some physiological parameters related to the cardiovascular and respiratory systems of workers who were exposed to heavy metal pollution. The study parameters in this work determine the degree of the harmful effect of metal pollution on the health of workers.

Materials and Methods

Subject: Inclusion and Exclusion Criteria

The present study includes 250 males and is divided into two groups: Group 1 (150 workers worked in different locations in the Erbil steel factory) and Group 2 (100 healthy males were used as a control group which was selected in the rural area away from the steel factory).

The mean ages of the subjects in all groups ranged between 25-45 years with no significant differences between the groups. The mean ages of the male control group were (35.28±5.26), while the mean ages of the workers were (38.78±6.53). The study has been conducted during the period from January 2021 to June 2022. The study excludes participants with smoking, drinking, diabetes, lung disease, hypertension, and any other illness that could affect the findings.

Collection of Samples

For each subject, 5 ml of blood was obtained by vein puncture. The blood sample was split into two parts of 2.5 ml. The first part was placed in a tube containing Ethylenediaminetetraacetic acid (EDTA) for a complete blood count (CBC). The second part was centrifuged at 300 rpm for ten minutes to collect the serum. The serum was stored at -20 degrees Celsius until it was used for the oxidative marker (MDA) and erythropoietin hormone measurement.

Measurement of the Serum MDA and Erythropoietin Hormone

A slightly modified spectrophotometric approach based on the interaction between MDA and thiobarbituric acid (TBA) was used to assess the level

of lipid peroxidation in the serum [19], at a wavelength of 532 nm with a molar extinction coefficient of ε532 = 1.56×10^5 M^{-1} cm^{-1}, absorbance was measured spectrophotometrically. Shortly the procedure included the addition of the 150 µL of serum to 1 ml of trichloroacetic acid 17.5% combined with 1 ml of 0.6% thiobarbituric acid. Vortex was thoroughly mixed with the mixture before it was put in a boiling water bath for 15 minutes and then allowed to cool. The mixture should then be added 1 ml of 70% trichloroacetic acid TCA, allowed to stand for 20 minutes at room temperature, centrifuged for 15 minutes at 2000 revolutions per minute, and the supernatant removed for spectrophotometric scanning. Regarding serum EPO, the commercially available human EPO ELISA was used to measure EPO. Human EPO ELISA Kit (ab274397) with 1 mIU/mL sensitivity to quantify human EPO.

Hematological Parameters

The RBC, Hb, hematocrit, Total WBC, lymphocytes, thrombocytes, and RDW were measured by using a CBC counter Convergys X3 of Technologies GmbH and Co.KG Germany.

Evaluation of the Cardiovascular and Respiratory Parameters

In the current investigation, a digital blood pressure monitor was used to measure blood pressure and heart rate. A pulse oximeter, an optical device that monitors the amount of oxygen-saturated hemoglobin in the tissue capillaries by passing a beam of light through the tissue to a receiver, was used to test the arterial oxygen level. The amount of air that can be forcibly expelled following a full inspiration, expressed in liters, is known as forced vital capacity (FVC). A basic spirometer is used to test vital capacity. Eupnea refers to a normal breathing rate, tachypnea refers to an elevated respiratory rate, and bradypnea refers to a lower-than-normal respiratory rate. When a person is at rest, the respiration rate is calculated by counting the number of breaths taken throughout a minute by counting how frequently the chest rises.

Table 1. Means±standard error of MDA and erythropoietin in control and different workplaces in the steel factory.

Workplace	MDA (µmol/L)	The concentration of serum erythropoietin (mU/mL)
Control (n = 100)	3.45±0.86 [d]	2.87±0.54 [c]
Managerial unit (n = 50)	5.24±0.98 [c]	8.35±1.02 [b]
Near the furnace (n = 50)	10.68±1.34 [a]	15.12±3.30 [a]
Scrap unit (n = 50)	7.78±1.56 [b]	10.30±2.15 [b]
p-value	0.001	0.001

Tukey's Post-Hoc-Test, no significant differences between the workplace working with the same letter. N = number of samples.

The Measurement of the Heavy Metals in Scalp Hair

A pre-coded polythene bag was rapidly filled with freshly cut human hair samples, sealed, and retained for pre-treatment. Using stainless steel scissors, the hair samples were reduced to 200-250 mg. After being cleaned in ethanol, the samples were coded and stored. According to a recommendation from the International Atomic Energy Agency, the stored samples were further divided into around 0.3 cm pieces, combined, and cleaned [20], first in ethanol, three times in distilled water, once more in ethanol, and then finally in distilled water. They were put in crucibles and dried for 15-25 minutes at 75ºC in the oven. The hairs were examined using an XRF (X-ray fluorescence spectrophotometer) from Sky Instrument Genius to determine the presence of Pb, Co, Hg, As, Mn, and Zn [21].

Statistical Analysis

Analysis of the data was performed using SPSS (Version 17). Results are expressed as means±standard error. The comparison of the studied parameters between different workplaces of working and years of working was done using analysis of variance (ANOVA) and Tukey's test. Pearson's correlation was used to find the correlation between the concentration of the heavy metals in the scalp hair of the workers with oxidative marker MDA and EPO hormone. A p-value equal to or less than 0.05 was considered to be statistically significant [22].

Results and Discussion

Oxidative Stress Marker and Erythropoietin Hormone

Comparison of the serum MDA and EPO hormone between healthy control and the workers who work in different units in the Erbil steel factory are presented in Table (1). The results found significant differences ($p\leq0.001$) between them. A higher concentration of the MDA (10.68±1.34 µmol/L) and EPO hormone (15.12±3.30 mU/mL) was observed in the serum of workers who work near the furnace part of the factory. The lower concentrations of MDA (3.45±0.86 µmol/L) and EPO (2.87±0.54 mU/mL) were seen in the control group. Moreover, the workers in the scrap unit

(7.78±1.56 µmol/L) showed higher MDA values when compared with that work in the manager unit (5.24±0.98 mU/mL). No significant differences in the EPO hormone were found between the workers in the scrap and manager unit.

Regarding the years of work which are presented in Table 2, the workers were divided into five groups according to the years of working in the steel factory: Group 1 (control); Group 2 (1-5 years working); Group 3 (6-10 years working); Group 4 (11-15 years working); and Group 5 (more than 16 years working). The obtained results showed significant differences in the MDA ($p \leq 0.001$) and EPO ($p \leq 0.01$) between the groups. The higher concentration of the MDA and EPO hormone was found in the groups of 11-15 years working (10.84±2.08 µmol/L MDA; 15.34±2.89 mU/mL EPO) and more than 16 years working (11.24±2.57 µmol/L MDA; 15.04±2.10 mU/mL EPO) when compared with the groups of control (3.45±0.86 µmol/L MDA; 2.87±0.54 mU/mL EPO), 1-5 years working (5.88±1.57 µmol/L MDA; 7.26 ±1.08 mU/mL EPO) and 6-10 years working (8.43±2.00 µmol/L MDA; 9.34±1.67 mU/mL EPO). The correlation between the concentration of the heavy metals in the scalp hair of the workers with oxidative marker MDA and EPO hormone is observed in Table 3. The results found a significant positive correlation between Pb ($r = 0.85 < 0.01$, Co ($r = 0.70 < 0.01$, Hg ($r = 0.74 < 0.01$ and As ($r = 0.60 < 0.05$) with MDA. No significant correlation was observed between heavy metals in the scalp hair and serum EPO hormones of the workers.

Massive increases in human exposure to heavy metals have been brought about by the industrial activities of the previous century. The most frequent heavy metals to cause human poisoning have been mercury, lead, chromium, and arsenic [23]. The chemicals that make up urban pollution are complex. There are various concentrations of gases like sulfide, carbon dioxide, carbon monoxide, ozone, and nitrogen dioxide. In addition to possibly aggravating the effects of particles, gases can have both immediate and long-term negative health effects [24]. Since a lot of these gases have oxidative characteristics, one possible way they can harm human health is by causing oxidative stress [25]. Reactive oxygen species (ROS) destroy biological elements like lipids, proteins, and DNA, and metals can cause oxidative stress by stimulating the production of these ROS [26, 27]. By assessing lipid peroxidation [28, 29], protein carbonylation [30], and DNA base oxidation [31], these oxidative damages can be identified. The total antioxidant capacity, which measures the overall effectiveness of cellular antioxidants against oxyradicals, as well as provides a thorough evaluation of responsiveness to oxidative stress [32], can be used to detoxify ROS by cellular antioxidant defense systems, both enzymatic and non-enzymatic [33].

By monitoring the generation of MDA, the degree of oxidative stress can be determined [34]. The recorded results in the present study found that the concentration of the MDA in the serum of the workers in the steel factory is more than two to three times that of the control groups. Also, the workers near the furnace have more MDA than the manager and scrap units of the factory. The workers around the furnace are more exposed to air pollution by gases and heavy metals when compared with the other parts. Many investigations agree with the present study and revealed a link between increased oxidative stress and exposure to heavy metals [35-37]. Heat stress is one of the most significant factors that might increase the generation of reactive oxygen species (ROS) [38]. So the increase in heat stress (high temperature) near the furnace parts is another factor that caused to increase in MDA oxidative biomarkers in the present study.

Table 2. Means±standard error MDA and erythropoietin of workers of different years working in the steel factory.

Years of working	MDA (µmol/L)	The concentration of serum erythropoietin (mU/mL)
Control (n = 100)	3.45±0.86 [d]	2.87±0.54 [d]
1-5 (n = 35)	5.88±1.57 [c]	7.26±1.08 [c]
6-10 (n = 40)	8.43±2.00 [b]	9.34±1.67 [b]
11-15 (n = 40)	10.84±2.08 [a]	15.34±2.89 [a]
16 and more (n = 35)	11.24±2.57 [a]	15.04±2.10 [a]
p-value	0.001	0.01

Tukey's Post-Hoc-Test, no differences between the years of working with the same letter. N = number of samples.

Table 3. Pearson's correlation (r) between the concentration of the heavy metals in the scalp hair, serum MDA, and EPO of the workers in the steel factory.

Metals (mg/kg)	MDA (µmol/L)	EPO (mU/mL)
Pb	0.85**	0.34
Co	0.70**	0.28
Hg	0.74**	0.30
As	0.60*	0.20
Mn	0.40	0.43
Zn	0.26	0.36

*Correlation is significant at 0.05 levels
**Correlation is significant at 0.01 levels.
A positive correlation was found between Pb, Co, Hg, and As in the scalp hair and serum MDA concentration of the workers.

Regarding the years of working in the steel factory, the concentration of MDA in the serum of workers is time-dependent. Our results found that ≥11 years of working as a higher MDA concentration in the serum of the workers, while 1-5 years have lower MDA. This means that the accumulation of the MDA in the serum is related to the years of exposure to the pollutants. The increasing of working years causes more oxidative stress marker MDA to persist in the body. This is approved by the foundation of the positive correlations between heavy metal accumulation in the scalp hair of the workers and serum MDA as mentioned in Table 3. Because of their higher metal storage and longer lifetimes, hair is used extensively in biomonitoring and as a biomarker for heavy metal pollution. Additionally, a lot of individuals want to provide samples, and it's simple to collect, store, and transport them [39]. So in the present study, the heavy metal concentration in the scalp hair was determined and correlated with the oxidative stress biomarkers, erythropoietin, and cardiovascular and respiratory system parameters as exposed to air pollution pollutants.

Hematological Parameters

A comparison of the hematological parameters between the control and the workers who work in different units in the Erbil steel factory is presented in Table 4. Significant differences in the RBC ($p \leq 0.001$), Hb ($p \leq 0.05$), hematocrit ($p \leq 0.001$), Total WBC ($p \leq 0.01$), lymphocytes ($p \leq 0.001$), and RDW ($p \leq 0.001$) were observed between the groups of workers in different units of the factory. The concentration of the RBC and Hb of the workers who work around the furnace (RBC = 4.646±0.066 $\times 10^6/\mu L$; Hb = 12.82±5.14 mg/dL) and scrap parts (RBC = 4.779±0.085$\times 10^6/\mu L$; Hb = 12.74±0.28 mg/dL) units of the factory are significantly lower than that of the managerial (RBC = 5.370±0.099$\times 10^6/\mu L$; Hb = 14.65±0.23 mg/dL) and control groups (RBC = 5.713±0.098$\times 10^6/\mu L$; Hb = 15.72±0.18 mg/dL). The workers in the scrap (42.47±0.89%), near the furnace (43.10±0.41%), and managerial (46.08±0.76%) units showed significantly lower hematocrit concentration when compared with the control group (49.05±0.58%).

The concentration of the total WBC and lymphocytes of the workers who work around the furnace (total WBC = 9.256±0.302$\times 10^3/\mu L$; lymphocytes = 39.15±1.26 %) and scrap parts (total WBC = 9.424 ±0.238$\times 10^3/\mu L$; lymphocytes = 36.91±1.25%) units of the factory are significantly higher than that of the managerial (total WBC = 7.300±0.307$\times 10^6/\mu L$; lymphocytes = 34.74±1.67%) and control groups (total WBC = 7.184±0.354$\times 10^6/\mu L$; lymphocytes = 30.13 ±1.88%). The workers in the scrap (36.82±0.65), near the furnace (35.47±0.62), and managerial (35.77±0.89) units showed significantly higher RDW when compared with the control group (17.15±0.45). No significant differences in platelet concentration were found between all the groups.

The results demonstrated in Table 5 showed the comparisons of the studied hematological parameters between the different years of working in the steel factory. The workers who worked more than six years and control showed significantly higher total WBC compared with those who worked for 1-5 years. Also the workers with 11 or more years working observed higher concentrations of lymphocytes compared with those with control, 1-5 and 6-10 years working. No significant differences in the RBC, Hb, hematocrit, platelets, and RDW were found between all years of working groups. While the control group significantly showed lower RDW than all years of working groups. The RBC, hematocrit, total WBC, lymphocytes, and platelets concentration in all worker groups are within the normal ranges of the World Health Organization guidelines. The decrease in the concentration of the Hb of workers in the furnace and scrap part of the Factory is under the normal ranges of the WHO guidelines. The increase in the lymphocytes and RDW of workers in all parts of the Factory and all working years is above the normal ranges of the WHO guidelines [40].

Table 4. Means±standard error of some hematological parameters in control and different workplaces of workers in the steel factory.

Workplace	RBC ($10^6/\mu L$)	Hb (mg/dL)	Hematocrit (%)	Total WBC ($10^3/\mu L$)	Lymphocytes (%)	Platelets ($10^3/\mu L$)	RDW
Control (n = 100)	5.713 [a] ±0.098	15.72 [a] ±0.18	49.05 [a] ±0.58	7.184 [b] ±0.354	30.13 [c] ±1.88	210.00 [a] ±8.25	17.15 [b] ±0.45
Managerial unit (n = 50)	5.370 [b] ±0.099	14.65 [a] ±0.23	46.08 [b] ±0.76	7.300 [b] ±0.307	34.74 [b] ±1.67	223.40 [a] ±9.50	35.77 [a] ±0.89
Near the furnace (n = 50)	4.646 [c] ±0.066	12.82 [b] ±5.14	43.10 [b] ±0.41	9.256 [a] ±0.302	39.15 [a] ±1.26	215.76 [a] ±7.98	35.47 [a] ±0.62
Scrap unit (n = 50)	4.779 [c] ±0.085	12.74 [b] ±0.28	42.47 [b] ±0.89	9.424 [a] ±0.238	36.91 [a] ±1.25	218.36 [a] ±10.72	36.82 [a] ±0.65
p-value	0.001	0.05	0.001	0.01	0.001	N.S.	0.001

Tukey's Post-Hoc-Test, no significant differences between workplaces working with the same letter. N= number of samples. N.S. = None-significant

Table 5. Means±standard error of some hematological parameters of workers of different years working in the steel factory.

Years of working	RBC (10^6/µL)	Hb (mg/dL)	Hematocrit (%)	Total WBC (10^3/µL)	Lymphocytes (%)	Platelets (10^3/µL)	RDW
Control (n = 100)	5.713 [a] ±0.098	15.72 [a] ±0.18	49.05 [a] ±0.58	7.184 [b] ±0.354	30.13 [c] ±1.88	210.00 [a] ±8.25	17.15 [b] ±0.45
1-5 (n = 35)	5.147 [a] ±0.085	14.40 [a] ±0.22	43.56 [a] ±0.60	6.665 [b] ±0.319	35.70 [b] ±1.31	221.86 [a] ±10.04	34.56 [a] ±0.49
6-10 (n = 44)	5.197 [a] ±0.084	17.74 [a] ±3.06	44.52 [a] ±0.60	7.547 [a] ±0.237	35.87 [b] ±1.14	211.88 [a] ±7.14	35.98 [a] ±0.65
11-15 (n = 40)	5.256 [a] ±0.106	14.63 [a] ±0.35	44.79 [a] ±1.06	7.745 [a] ±0.415	38.21 [a] ±2.02	201.36 [a] ±12.45	35.66 [a] ±1.17
16 and more (n = 35)	5.146 [a] ±0.417	14.72 [a] ±0.19	44.62 [a] ±0.65	7.858 [a] ±0.262	38.93 [a] ±1.52	221.62 [a] ±8.51	36.84 [a] ±0.77
p-value	N.S.	N.S.	N.S.	0.05	0.05	N.S.	0.046

Tukey's Post-Hoc-Test, no differences between the years of working with the same letter. N = number of samples.
N.S. = None-significant

Information on the quantity and distribution of blood cells is available from hematological tests. With the help of this test, pathologic disorders like anemia can be diagnosed by learning more about the types and amounts of blood cells, including RBC, WBC, and platelets [41]. To maintain oxygen homeostasis, EPO is produced in the kidney and liver in a hypoxia-inducible manner by activating hypoxia-inducible transcription factors. One viable treatment approach for addressing anemia brought on by renal disorders involves accelerating EPO synthesis in hepatocytes [42]. The main physiological trigger for EPO synthesis is hypoxia, which, depending on the severity of hypoxia, can elevate blood EPO levels by up to several folds [43]. An adult human's kidneys produce the peptide hormone known as human EPO. It controls bone marrow erythropoiesis as a key hematopoietic growth factor by encouraging the daily huge creation of RBCs with hemoglobin A, which carry oxygen and deliver it to tissues [44, 45].

A complete blood count is a test that reveals details about the quantity and distribution of blood cells. This examination offers important data on the types and amounts of blood cells, including RBC, WBC, and platelets, which alerts medical practitioners to look for symptoms like weakness, exhaustion, or bruising. Additionally, it diagnoses pathological disorders like anemia [41]. The decreases in the RBC and hemoglobin concentrations of workers exposed to different pollutants in the present study are consistent with [46] who found that exposure to air pollution was strongly linked to an increase in the prevalence of anemia and a drop in hemoglobin levels in an older American sample. Also [47] revealed that the prevalence of anemia was higher in children who were exposed to higher levels of air pollution. According to the study of [48], workers exposed to pollutants at gas stations in north Ethiopia experienced a decline in total RBC count, Hg content, and HCT value as exposure time rose from less than 2 years to more than 8 years.

The platelet count in the present study has not changed and these findings are different from those of [48], who observed a decrease in the platelet count in the workers exposed to pollutants. Exposure to pollutants has a time-dependent effect. Long-term exposure to an agent without protection may permanently inhibit bone marrow function and reduce the production of new blood cells, which can result in aplastic anemia [49]. Shortened RBC life and impairment of heme production by the metabolic byproduct of free radicals which are produced by pollutants may be responsible for decreases in hemoglobin content and RBC count. These free radicals can change how heme proteins are produced in the bone marrow and the erythrocyte membrane [50]. In the present study due to the decrease in the RBC and hemoglobin concentration of the workers exposed to pollution in the steel factory, the EPO hormone increases. The EPO enhances the bone marrow to increase the production of the RBC and Hb. A negative correlation between Pb concentration and Hb and a positive correlation between Pb and RDW was observed [51], these results are in line with our findings which revealed that the workers in the polluted sites of the steel factory showed lower concentrations of RBC and Hb with a higher RDW when compared with the control group.

A considerable increase in the median RDW value of workers in the polluted sites as compared to healthy controls was also revealed by our findings. The primary sign RDW primarily shows aberrant red blood cell breakdown and poor erythropoiesis. Although it may be brought on by pollutants, it is also linked to allergic reactions and inflammation. RDW is a reliable indicator of mortality from all causes, including heart disease, and cancer [52]. In the literature, it has been shown that air pollution is linked to health risks and that disease processes may be significantly influenced by inflammatory responses. One indicator of inflammation is the WBC count, but the literature offers conflicting

views on the connection between WBC counts and exposure to air pollution [53]. The increase in the total WBC and lymphocytes in the present study is consistence with the finding of [54] who observed a time-dependent increase in the total WBC and lymphocytes in healthy Netherlands subjects exposed to air pollutants. Also [53] found the same results in the healthy Taiwan subjects. An enhanced immunological response has been linked to heavy metal buildup in the body. Increased WBC was substantially correlated with heavy metals such as Pb, As, Cu, and Cd [55].

Cardiovascular and Respiratory Parameters

Cardiovascular and respiratory parameters of the control and workers are investigated in Table 6. The obtained results found a significant difference in the SBP ($p \leq 0.05$), heart rate ($p \leq 0.05$), FVC ($p \leq 0.001$), and breathing rate ($p \leq 0.001$) between the groups. The workers in the furnace and scrap parts of the factory showed a higher SBP (144.76±4.06 mmHg; 132.88±3.46 mmHg) and heart rate (85.44±2.09 beat/minute; 84.32±2.55 beat/minute) respectively than that of the manager (126.96±3.55 mmHg; 77.00±2.36 beat/minute) and control (122.92±2.07 mmHg; 77.08±1.80 beat/minute) groups respectively. A significantly lower FVC of the workers was recorded in the furnace (1980.00±93.80 ml) and scrap (2188.00 ±91.52 ml) units in the factory, while the control group showed a higher FVC (3234.00±104.19 ml). The breathing rate of the control group (16.32±0.63 breath/minute) is lower than that of the other workers in the manager (21.32±0.64 breath/minute), furnace (22.04 ±0.63 breath/minute) and scrap (22.88±0.69 breath/minute) units. No significant differences in the DBP and saturation of the blood with oxygen in the artery were found between all the groups.

As shown in Table 7, 16 and more years of working results a significantly ($p \leq 0.001$) higher SBP (148.95±5.02 mmHg) and lower FVC (2037.5±110.44 ml), while the control and 1-5 years of working showed a lower SBP (122.92±2.07 mmHg for control and 122.95±1.52 mmHg for 1-5 years working) and a higher FVC (3234.00±104.19 ml for control and 2465.21±98.78 ml for 1-5 years working) when compared with 6-10 and 11-15 years working. Also, 11-15 working years results in higher SBP (138.45±2.60 mmHg) as compared with the 6-15 working years (126.05±2.33 mmHg). The DBP of the workers who work 11≥years in the steel factory is significantly higher than those working for ≤6 years. No significant differences in the heart rate, arterial oxygen saturation, and breathing rate between all years of working are observed. While the control group significantly showed lower breathing rates than all years of working groups.

A significant amount of illness and mortality linked to air pollution is caused by the cardiovascular consequences of inhaled particulate matter (PM) [56]. Over 90% of people worldwide are thought to reside in places that exceed World Health Organization (WHO) air quality standards [57]. It has been calculated that bringing global air pollution under the WHO air quality criteria would lengthen life expectancy by 0.6 years, providing benefits comparable to those of curing lung and breast cancer [58]. The relationship between air pollution and respiratory illnesses including chronic obstructive pulmonary disease and asthma has long been documented. Furthermore, in the twenty-first century, it is well acknowledged that air pollution is linked to cardiovascular illness, a finding that was originally startling. Premature mortality from air pollution is caused by cardiovascular diseases in the range of 40-60% [59-61].

In our study, significant increases in the systolic blood pressure and heart rate of the workers were observed particularly those found near the furnace and scrap part of the factory. The key role between increased blood pressure and air pollution is oxidative

Table 6. Means±standard error of some cardiovascular and respiratory system parameters in control and different workplaces of workers in the steel factory.

Workplace	SBP (mmHg)	DBP (mmHg)	Heart rate (Beat/minute)	Arterial blood oxygen (%)	FVC (ml)	Breathing rate (Breath/minute)
Control (n = 100)	122.92 ^{c} ±2.07	79.04 ^{a} ±1.59	77.08 ^{b} ±1.80	96.28 ^{a} ±0.71	3234.00 ^{a} ±104.19	16.32 ^{b} ±0.63
Managerial unit (n = 50)	126.96 ^{c} ±3.55	79.18 ^{a} ±2.34	77.00 ^{b} ±2.36	97.84 ^{a} ±0.17	2516.00 ^{b} ±151.73	21.32 ^{a} ±0.64
Near the Furnace (n = 50)	144.76 ^{a} ±4.06	83.68 ^{a} ±3.38	85.44 ^{a} ±2.09	97.12 ^{a} ±0.47	1980.00 ^{c} ±93.80	22.04 ^{a} ±0.63
Scrap part (n = 50)	132.88 ^{b} ±3.46	80.40 ^{a} ±2.14	84.32 ^{a} ±2.55	97.24 ^{a} ±0.42	2188.00 ^{c} ±91.52	22.88 ^{a} ±0.69
p-value	0.05	N.S.	0.05	N.S.	0.001	0.001

Tukey's Post-Hoc-Test, no significant differences between workplaces working with the same letter. N = number of samples.
N.S. = None-significant

Table 7. Means±standard error of some cardiovascular and respiratory system parameters of workers of different years working in the steel factory.

Years of working	SBP (mmHg)	DBP (mmHg)	Heart rate (Beat/minute)	Arterial blood oxygen (%)	FVC (ml)	Breathing rate (Breath/minute)
Control (n = 100)	122.92 ^{c} ±2.07	79.04 ^{b} ±1.59	77.08 ^{a} ±1.80	96.28 ^{a} ±0.71	3234.00 ^{a} ±104.19	16.32 ^{b} ±0.63
1-5 (n = 35)	122.95 ^{c} ±1.52	74.39 ^{b} ±1.63	73.65 ^{a} ±1.91	97.56 ^{a} ±0.16	2465.21 ^{b} ±98.78	20.97 ^{a} ±0.70
6-10 (n = 40)	126.05 ^{c} ±2.33	78.58 ^{b} ±2.01	77.02 ^{a} ±1.52	97.66 ^{a} ±0.25	2290.04 ^{c} ±102.31	20.97 ^{a} ±0.48
11-15 (n = 40)	138.45 ^{b} ±2.60	82.90 ^{a} ±1.30	77.09 ^{a} ±3.33	96.90 ^{a} ±0.63	2201.90 ^{c} ±105.48	21.09 ^{a} ±1.30
16 and more (n = 35)	148.95 ^{a} ±5.02	86.20 ^{a} ±3.06	82.58 ^{a} ±2.94	97.08 ^{a} ±0.46	2037.50 ^{d} ±110.44	21.54 ^{a} ±0.70
p-value	0.001	0.007	N.S.	N.S.	0.001	0.044

Tukey's Post-Hoc-Test, no significant differences between the years of working with the same letter. N= number of samples.
N.S. = None-significant

stress. As mentioned earlier, the serum concentration of the oxidative marker MDA of the workers especially those working at the furnace and scrap part in our study is significantly higher than that of the control one. Other investigators approved the role of oxidative stress that results from air pollution and its effects on the cardiovascular system [62, 63]. Strong evidence for oxidative pathways has been provided by epidemiological research and controlled exposure studies in humans, and a network of mechanistic studies in animals and cellular models has expanded on these findings [64].

Exposure to PM has frequently been linked to modest but considerable increases in blood pressure [65-67]. The biological processes that contribute to the hypertensive effect of air pollution, as well as other air pollutants, are thought to be complex [68]. It has been hypothesized that oxidative stress plays a role in the pattern of vascular dysfunction brought on by PM. Nitric oxide (NO), a significant mediator generated from endothelial cells that regulates blood channel vasodilatation, is scavenged by the oxygen-free radical superoxide. The endothelium-dependent flow-mediated dilatation as well as the endothelium-independent NO-releasing vasodilation to nitroglycerin were both impaired by PM2.5 exposure. Oxidative stress may be the cause of this pattern of impairment (inhibition of vasodilators acting through NO) [69].

The decrease in the lung function (FVC) of the workers particularly those who work near the furnace and scrap part of the steel factory in our study is in agreement with [18] who revealed that heavy metals, especially mercury and manganese, were linked to impaired lung function regardless of single or combined exposure in northwest China. According to a study conducted in 112 US communities, every 10 g/m^3 increase in PM2.5 concentration was associated with a 1.68% increase in respiratory illness mortality [70]. The death rate from respiratory disorders increased by 1.51% for every 10 g/m^3 increase in PM2.5 concentration, according to a systematic review and meta-analysis of 110 time-series studies carried out in various parts of the world [71]. Additionally, a study from Latin America found a 2% increase in the chance of dying from cardiovascular and respiratory disorders for every 10 g/m^3 increase in PM2.5 concentration, which is consistent with research from Europe and North America [72].

Conclusions

The current study concluded that air pollutants resulting from the Erbil steel factory negatively impact on cardiovascular and respiratory systems of the workers. The oxidative marker, blood pressure, and heart rate increased in the workers by increasing the workplace and years of exposure to the pollutant. Lung function and hematological parameters are decreased also according to the workplace and years of working. The most effects are seen in the furnace and scrap parts of the factory and it is due to high exposure of the workers to the pollutants in these parts.

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Conflict of Interest

The authors declare no conflict of interest.

Human Ethics Declaration

The samples of the study were taken following the Helsinki Declaration of 1975, as revised in 2000 and approved by the Human Ethical Committee of Salahaddin University, College of Science, Biology department, and numbered sheet 321/4S issued on 28/3/2021.

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