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ORIGINAL ARTICLE

Effects of SO_2, Ozone, and Ambient Air Pollution on Iron, TIBC, and Hematological Parameters in a Rat Model

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KEYWORDS

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ABSTRACT: With urbanization and industrialization, air pollution is one of the known health hazards and a severe problem in human societies. This study assessed the relationship of SO_2, O_3, and ambient air pollution on hematologic factors, serum iron, and TIBC in a rat model. A total of 32 male Wistar rats were enrolled in the study. They were randomly divided into the four study groups (n = 8 in each) as follows: The control, SO_2 group exposed to 10 ppm, ozone group (O_3) exposed to 0.6 ± 0.1 ppm, and ambient air pollution (AAP) group for five weeks (3 h day^{-1}). One-way ANOVA (analysis of variance) was used to compare parameters. Significant changes in RBC (P=0.026), hemoglobin concentration (P=0.029), mean corpuscular volume (MCV) (P=0.011), mean corpuscular hemoglobin concentration (MCHC) (P<0.001), monocytes (P=0.002), and basophils (P=0.022) were observed between control and AAP groups. The other parameters showed an insignificant difference for these two groups' comparison. In the of leucocytes' differential counts, only basophils and monocytes were statistically high in the AAP group compared to the control group (P=0.002 and P=0.022, respectively). In terms of serum iron, the differences between the control and SO_2 groups (P =0.008), or between the groups of O_3 and APP (p=0.028) were statistically significant. The observed hematological and biochemical changes indicated the toxic effects of ambient air particles. Further epidemiological studies are necessary to investigate the impact of other air pollutants like NO_2, NO, and CO on hematological and biochemical parameters.

INTRODUCTION

Air pollution is a combination of hazardous materials from both human-made and natural resources. Exposure to air pollution is associated with oxidative stress and inflammation in human cells, predisposing them to many diseases[1]. Both acute and chronic exposure to air pollution are the risk factors for cardiovascular function

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impairment, exacerbate underlying disease, and increase cardiovascular mortality. However, there is incomplete knowledge about the exact plausible biological mechanisms of these associations[2]. Air pollutants are a mixture of particulate matter (PM) and gases, including nitrogen dioxide (NOx), ozone (O_3), sulfur dioxide (SO_2), polycyclic aromatic hydrocarbons, and volatile organic compounds. PM with an aerodynamic diameter ≤2.5μm ($PM_{2.5}$) has a small molecular diameter and large surface area. Therefore, they absorb heavy metals, enter the deeper respiratory tract, and cross the lung capillary network. Finally, it leads to severe damage to various body systems [3].

Previous studies reported the role of PM on atherogenesis, pro-inflammatory state related to cardiometabolic risk factors, and hematological parameters[4]. In terms of hematological parameters, studies demonstrated that the elevation of white blood cells (WBC)[5,6], red blood cells (RBC)[5], and platelets (PLT)[6] lead to an increment in the odds ratio of clustering of cardio-metabolic risk factors [5]. Meanwhile, the relationship between air pollution and hematological changes remains unclear, and there is a controversy in the published studies in this regard. Some studies reported the association of air pollution exposure with WBC count[7], while others refuse it[8]. There are limited studies on the association of air pollutants and hematologic factors, as well as serum iron and total iron bind capacity (TIBC).

Due to availability, cost, and ability to examine the genetically modified strains, animal models have been extensively used to evaluate the association between environmental exposure to particulate air pollutants and cardiovascular and respiratory diseases and their underlying mechanisms [9]. This study assessed the relationship of SO_2, O_3, and ambient air pollution on hematologic factors, serum iron, and TIBC in a rat model.

MATERIALS AND METHODS

Animals, experimental design, and exposure

Thirty-two male Wistar rats (weighing 200 ± 20g) were housed under standard conditions (22 ± 2°C, 12/12 h light/dark cycle, and 50–70% humidity). They were randomly divided into the four study groups (n = 8 in each) as follows: The control, SO_2 group, ozone group (O_3), and ambient air pollution (AAP) group. The rats in the control group didn't receive any air pollutants and were only placed under filtered air conditions. Rats in the SO_2 group were placed for five weeks (3 h day^{-1}) in 10 ppm SO_2. In the chamber, the mixed gas composition was 0.002% SO_2, 20.5% O_2, and 79.5% N, with 1 l min^{-1} flow rate of. Researchers measured the chamber SO_2 concentration daily using a GASTEC SO_2 detector tube (No.5La) with the measuring range of 2-30 ppm.

In the O_3 group, the rats were placed in a chamber house with 0.6 ± 0.1 ppm 3 h day^{-1} for five weeks. An O_3 producer system provided the O_3, and its concentration was measured by an O_3 detector (model: A-21ZX-USA) daily.

Finally, the rats in the AAP group were put in a busy city site for 3 h day^{-1} for five weeks. It is at the city center square (Abresan) of Tabriz in East Azerbaijan of Iran with high-traffic. That is located near the station of air pollution recorder.

Biochemical analyses

After twenty hours of the last exposure period, animals were anesthetized and blood samples were obtained by cardiac puncture under anesthetic condition (Ketamine/Xylazine, 60/10 mg kg^{-1} IP) [10] and transferred into tubes containing ethylene-diamine-tetra acetic acid (EDTA) and clot tubes for hematological and biochemical analyses. The clot tubes were centrifuged (3000 g, 10 min) and the blood serum samples were separated and placed in a deep freezer (-70°C). Commercial biochemical kits (Pars Azmun, Karaj, Iran) with enzymatic methods and an automated biochemistry analyzer (Abbott, Alcyon 300, USA) were used for biochemical analysis (iron and TIBC).

Hematological analysis

The hematological parameters were detected by Mindray Hematology Analyzer (Shenzhen, P. R. China).

Statistical analyses

Data normality was analyzed by the Kolmogorov-Smirnov test. Then, statistical analyses were performed

using Windows SPSS 16. The comparison between the groups and multiple comparisons were done using one-way ANOVA (analysis of variance) and Tukey post-hoc test, respectively. The data were reported as mean ± 1 SD (standard deviation). P-values < 0.05 were significant.

RESULTS AND DISCUSSION

AAP monitoring

Table 1 shows the report of the Iranian AP Monitoring System in the period of the study.

Table 1. The mean concentrations of AAP parameters for Tabriz Abresan square during the study reported by the Iranian Air Pollution monitoring agency (mean ± SD). http://aqms.doe.ir/Home/AQI

Parameter		SO_2	CO	NO_2	O_3	$PM_{2.5}$	PM_{10}
Ambient air concentration (μg m^{3-1})		2.00 ± 1.17	14.08 ± 0.07	23.96 ±8.26	49.76 ± 18.48	28.67 ±4.04	21.33 ±5.13
WHO Guideline Values (μg m^{3-1})	10-minute mean	500	-	-	-	-	-
	1-hour mean	-	-	200	-	-	-
	8-hour mean	-	-	-	100	-	-
	24-hour mean	20	-	-	-	25	50
	annual mean	-	-	40	-	10	20

Changes in hematological parameters after AAP exposure

Our results showed significant differences in RBC (P=0.026), hemoglobin concentration (P=0.029), MCV (mean corpuscular volume) (P=0.011), MCHC (mean corpuscular hemoglobin concentration) (P<0.001), monocytes (P=0.002), and basophils (P=0.022) between control and AAP groups (Table 2). In leucocytes' differential counts, only monocytes and basophils were statistically high in the AAP group compared to the control group (P=0.002 and P=0.022, respectively). There were no statistical differences between hematocrit, platelets, plateletcrit (PCT), and mean platelet volume (MPV) in any comparison between study groups.

Although the RBC and hemoglobin levels were decreased in all intervention groups in comparison with the control group, it was only statistically different in the AAP group.

Table 2. Changes in hematological parameters in different study groups.

Parameters	Control (n=8)	SO_2 (n=8)	O_3 (n=7)	APP (n=8)	p-value
RBC (*$10^6$$mm^{3-1}$)	9.47 (0.96)	8.99 (0.60)	8.70 (0.38)	8.52 (0.31)	0.026in comparison between the control and AAP group
Hb (g l^{-1})	16.1 (1.75)	14.89 (0.77)	15.27 (0.81)	14.50 (0.47)	0.029 in comparison between the control and AAP group
Hct (%)	48.55 (5.31)	44.50 (2.82)	46.52 (2.74)	45.71 (1.89)	P>0.05 in all comparisons
MCV (fl)	51.05 (0.27)	49.56 (1.80)	53.47 (1.71)	53.81 (1.23)	0.002 in comparison between the control and AAP group 0.011 in comparison between the control and O_3 groups
MCH (pg)	16.95 (0.53)	16.60 (0.56)	17.47 (0.26)	17.06 (0.28)	0.003 in comparison between SO_2 and O_3 groups
MCHC	33.17 (0.40)	33.48 (0.55)	32.86 (0.69)	31.73 (0.64)	<0.001 in comparison between control, SO_2 groups, and AAP group 0.004 in comparison between O_3 and AAP groups 0.007 in comparison between the control and SO_2 group
RDW (%)	16.31 (0.42)	15.40 (0.35)	16.54 (0.60)	16.45 (0.62)	0.001 in comparison between the SO_2 and O_3 group 0.002 in comparison between SO_2 and AAP group
WBC (*10^3 μl^{-1})	14.49 (6.27)	14.08 (5.61)	10.71 (3.26)	14.87 (7.16)	P>0.05 in all comparisons

NEUT (%)	9.26 (5.33)	7.87 (4.65)	5.41 (2.74)	8.66 (1.36)	P>0.05 in all comparisons
LYMP (%)	4.89 (2.27)	5.70 (2.20)	4.90 (2.22)	5.80 (4.56)	P>0.05 in all comparisons
MONO (%)	0.09 (0.08)	0.06 (0.05)	0.14 (0.05)	0.34 (0.21)	0.002 in comparison between the control and AAP group 0.001 in comparison between SO_2 and AAP group 0.027 in comparison between the O_3 and AAP group
EOS (%)	0.13 (0.05)	0.33 (0.23)	0.17 (0.08)	0.14 (0.09)	0.032 in comparison between the control and SO2 group 0.049 in comparison between SO_2 and AAP group 0.022 in comparison between the control and AAP group
Baso (%)	0.15 (0.08)	0.13 (0.07)	0.14 (0.05)	0.25 (0.05)	0.003 in comparison between SO_2 and AAP group 0.017 in comparison between the O_3 and AAP group
PLT (*10^3 μl^{-1})	669.12 (74.47)	692.62 (78.65)	643.71 (47.40)	647.75 (57.70)	P>0.05 in all comparisons
PCT (%)	0.39 (0.06)	0.39 (0.05)	0.35 (0.03)	0.37 (0.04)	P>0.05 in all comparisons
MPV (fl)	5.80 (0.14)	5.63 (0.15)	5.60 (0.10)	5.74 (0.20)	P>0.05 in all comparisons

Data are presented as mean ± 1 SD; RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: Red Blood Cell Distribution Width; WBC: white blood cell; NEUT: Neutrophils; LYMP: Lymphocytes; MONO: Monocytes; EOS: Eosinophils; Baso: Basophils; PLT: platelets; PCT: plateletcrit; MPV: Mean Platelet Volume

Changes in hematological parameters after a SO_2 and O_3 exposure

Our results demonstrated that MCV in the SO_2 group was less than the others, and in the O_3 groups, was similar to the value of the AAP group. There are significant differences between the control and O_3 groups (P=0.011), SO_2 with O_3 groups (P<0.001), and SO_2 with AAP group (P=0.003). The mean corpuscular hemoglobin (MCH) level in the O_3 group was similar to the AAP exposure rats. The level of MCH in the control and SO_2 groups was less than that in the AAP and O_3 groups. The results showed significant differences between SO_2 and O_3 groups (P=0.003).

MCHC in the control and SO_2 groups was higher than the AAP and O_3 groups. However, only there was a significant difference between O_3 and AAP groups (P=0.004) and SO_2 with AAP exposure rats (P<0.001).

RDW in the SO_2 group was lower than the others, and it was statistically different from the control (P=0.007), O_3 exposure rats (P=0.001), and AAP group (P=0.002).

WBC in the O_3 group was lower than in other groups, but this difference was not statistically significant.

Both control and SO_2 exposure rats had lower monocytes with a significant difference between O_3 and AAP groups (P=0.027) or between SO_2 and AAP rats (P=0.002). A similar association was observed in basophils (P=0.017), while its amount in the control group was higher than in the O_3 group. SO_2-exposure rats had a lower amount of basophil than the AAP group (P=0.003). Regarding eosinophils, the highest amount belonged to the SO_2 group, and there was an association between the amount of them in the SO_2 group and control rats (P=0.032) and between the SO_2 and AAP group (P=0.049).

Changes in biochemical parameters

The control group's mean iron level was lower than the others. Our findings revealed a statistically significant difference between the control and SO_2 groups (P =0.008) or between O_3 and APP groups (p=0.028) (Figure 1). In addition, the mean TIBC level was high in all air pollution groups. However, this difference was not statistically significant (P>0.05) (Figure 2).

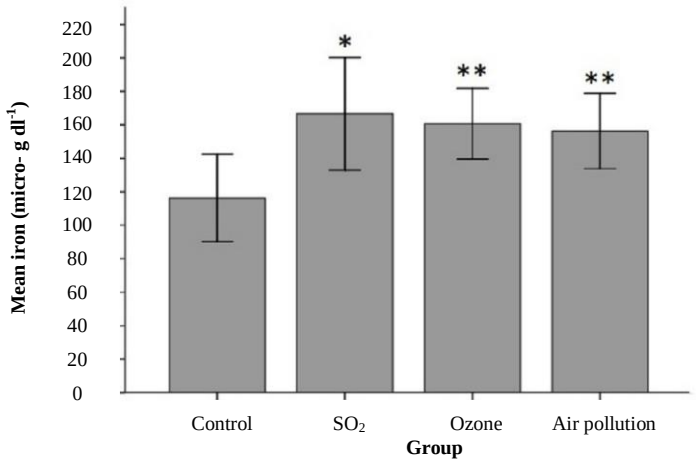

Figure 1. Effects of SO_2, ozone, and ambient air pollution on blood serum iron of the rats (n = 8). *and **: statistically significant difference compared to control group ($P < 0.01$ and $p<0.05$, respectively).

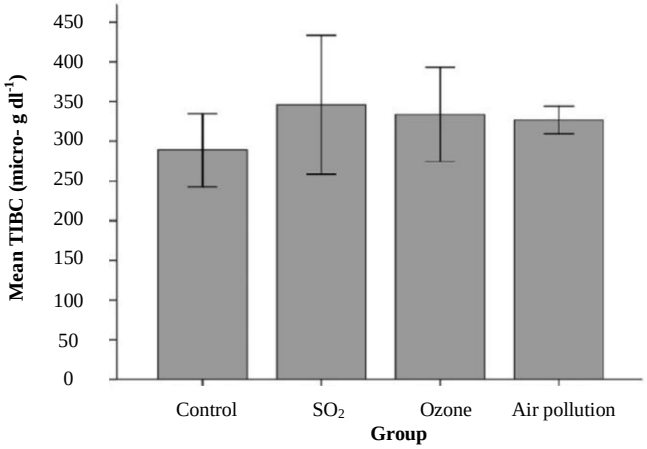

Figure 2. Effects of SO_2, ozone, and AAP on blood serum TIBC of the rats (n = 8).

Effect on hematological parameters

Air pollution, especially PM, has a significant impact on the body's health with an unclear biological acting mechanism[2]. The evidence suggests that poor hematological profile may be associated with exposure to high levels of AAP. A study on individuals after exercise in highly polluted air showed that RBC count, hemoglobin, PCV, and MCHC are decreased, possibly due to a slight increase in blood volume after exposure to high levels of air pollutants [11].

CBC is a parameter to evaluate the health condition with various responses to age, gender, and genetic background [12]. However, the changes in WBC may be an index for diseases, whereas RBC, besides hematocrit and hemoglobin concentrations, is an index of blood oxygen transfer capacity [13]. The present study has presented the results of the AAP exposure impact for five weeks on hematological parameters. We could find a difference between the level of RBC, hemoglobin, MCV, MCHC, monocytes, and basophil between the control and APP groups.

Long-term exposures to PM and NO_2 are associated with decreased hemoglobin levels and increased prevalence of anemia in older adults in China [14]. The other studies showed similar results, in line with our investigations. Another study revealed that long-term NO_2 and SO_2 exposure leads to lower hemoglobin levels and RBC counts than those children who lived in less polluted areas[15]. The outcomes of short-term AP exposures on hemoglobin levels are inconsistent. Some studies confirmed the decrease in hemoglobin levels in PM_{10}

exposure, or PM_{10}, NO_2, and SO_2 [16]. Contrast results were reported with exposure of $PM_{2.5}$ in women and not in men or vice versa in men rather than women.

Air pollution may decrease the kidney's erythropoietin secretion and increase endogenous erythropoietin resistance in the bone marrow, reducing RBC production and lowering hemoglobin levels [17]. AP can induce IL-6 gene expression in alveolar macrophages, too. It upregulates hepatic hepcidin production [18] and increases hepcidin, an iron regulatory protein that decreases gastrointestinal iron absorption and leads to anemia[19].

Fauzie et al. [20] exposed mice for five days and three months and revealed an insignificance decrease of RBC and hemoglobin as well as an increment of WBC on five days of exposure. Similar results were observed on three months of exposure, except for WBC, which showed a reduction compared to the control group. Our results are in line with the mentioned studies.

In terms of WBC differential count, Fauzie et al. [20] showed a decrease in eosinophil and lymphocytes in 5 days exposure group compared to the control group and an increment of these parameters in 3 months exposure and monocytes in both short-term and short-term medium exposure. However, none of the hematological parameters didn't differ statistically significantly ($P>0.05$). In the point of neutrophils, only medium-term exposure was accompanied by a statistical decrease compared to control group ($P=0.047$). The opposite result was observed in the short-term exposure was observed. In our study, the number of neutrophils was low in all interventions compared to the control group, despite its insignificance difference. Our results are in in the order of some previous studies and support the findings of other reports, too[21].

Goto et al., in a study on atherosclerotic rabbits, demonstrated an increment release of monocytes from the bone marrow, which was accelerated by PM_{10} exposure [22]. Their results are in accordance with ours.

The association is reported with exposure of PM_{10} and WBC counts, so the WBC count has been increased [23], which might be due to the tissue-damaging effects and rise of antibody production in response to the exposure.

Steenhof et al. evaluated the effect of PM_{10} exposure at different time points and revealed a positive association between WBC count and neutrophil, whereas they didn't find this association with monocytes. They concluded that short-term air pollution exposure could induce WBC count changes in healthy individuals [24]. Their results are not in line with ours, which may be due to the different exposure times. Bruske et al. investigated PM's effect and variables gaseous, including SO_2, NO_2, NO, CO, and O_3, on hematological parameters in patients with chronic pulmonary diseases. They found that polymorphonuclear leukocytes have been decreased. Lymphocytes increased within 24 hours in association with all gaseous pollutants without any change regarding PM. Monocytes were increased in response to ultrafine particles and NO[25].

Our results showed that the level of MCV was low in SO_2 exposure and high in both O_3 and AAP groups compared to the control group. MCH was high in O_3 and AAP exposure rats rather than control and SO_2 groups. The MCHC was similar in control and SO_2 exposure rats, whereas it was low in O_3 and AAP groups. MCH indicates the capacity of oxygen-carrying hemoglobin in the blood, and MCHC indicates hemoglobin concentration within RBCs. When the MCHC level is low, the hemoglobin concentration in the RBC has been reduced.

Rudež et al. showed an association between air pollution (PM < 10 μm, CO, NO, NO_2, and O_3) on platelet aggregation and thrombin generation except for O_3 ($P < 0.01$ and < 0.05, respectively) [26]. We couldn't find any association between air pollutant exposure and platelet level in the study groups.

Effects on biochemical parameters

The iron hemostasis undergoes changes in response to air pollutants. These changes are made via the pollutant or metabolite capacity, complex or chelate iron from pivotal sites in the cell, or pollutant ability to displace the iron from pivotal sites in the cell [27]. The serum iron level is a biomarker for environmental iron-chelating chemicals exposure.

The serum iron concentration refers to the total amount of iron in serum, and TIBC indicates the maximum amount of iron required to bind to all the transferrin in

serum. In low serum iron, the TIBC increases, and vice versa [28]. Exposure to environmental pollutants affects the acute phase response indices, serum iron levels, and RBC production. The acute phase reaction involves the loss of functional iron, which, if continued, can lead to anemia[29]. In the present study, we found an association between serum iron levels and air pollutant exposure in different study groups, while we didn't observe such association in TIBC levels. Various studies evaluated air pollution's effect on the induction of iron deficiency anemia and reported a positive association. At the same time, we observed that the serum level was high in air pollutants exposure groups.

Besides the strengths of the current study in measuring the level of serum iron and TIBC, the hematological factors, and the duration of exposure in different groups, our study had some limitations, including the exposure of some air pollutants on rodents rather than the other essential pollutants. Further epidemiological studies are necessary to investigate the impact of other air pollutants like NO_2, NO, and CO on hematological and biochemical parameters.

CONCLUSIONS

The current study demonstrated the importance of attention to air pollutants on the hematological and biochemical parameters using small rodents as objects of study. The airborne PM induced changes in blood parameters, including RBC, hemoglobin, MCV, MCHC, monocytes, basophil, and serum iron levels. All of the mentioned items were high in the AAP group except RBC, hemoglobin, and MCHC compared to the control group. In conclution, the observed hematological and biochemical changes indicated the toxic effects of ambient particles that the study animals have inhaled. More investigations with different exposure periods and related variables, such as blood gases, are recommended.

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ETHICAL CONSIDERATION

The experiments were performed according to the guidelines of the research ethics committee of Tabriz University of Medical Sciences (Ethics approval code: IR.TBZMED.VCR.REC.1399.037).

Conflict of interests

The authors declare no conflicts of interest.

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