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Seasonal And Regional Effects of Air Quality Index on Hematological Indices of Dogs Under Local Environmental Conditions In Pakistan

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Abstract: Air pollution is found to have significant association with living health all over global world. Environment Protection Department of Punjab, Pakistan provides the monthly air quality index (AQI) data on air pollution with concentrated particulates like $PM_{2.5}$, PM_{10}, NO_2, SO_2 and O_3. Air particulates concentrations may vary of season, regional geography and climate. We performed blood sampling of 45 dogs from different breeds (Labrador retrievers, German shepherds and Pit bulls) from three different areas categorized on basis of AQI as less polluted (Gulberg), polluted (Town Hall) and highly polluted (Shadman) in winter and spring seasons. Data were analysed using paired sample t-tests for seasonal study and independent sample t-tests for area and breed study by SPSS ($P < 0.05$ ascertained as significant). Seasonal study resulted that less polluted area had no significant effects on hematological indices in any three breeds of dogs in both seasons. Polluted area was presented with increased significant effects on values of monocytes in Labrador retrievers, Hct and MCHC in German shepherds while decreased significant effects on MCH value in Pit bulls in winter season as compared to spring season. Highly polluted area had significant effects with decreasing WBC counts only in German shepherds in winter season than spring season. This study concludes that polluted and highly polluted areas in winter season with worst AQI affects blood indices more than spring season in dogs.

Keywords: Air pollution, hematological indices, seasonal variations, dogs.

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Introduction

Air pollution is a global issue in this era and many countries are putting efforts to curb this problem. Air pollution is thought to have deleterious impacts on quality of life [1]. According to world air quality reported data, Pakistan is found to be most polluted country in 2018. Lahore the capital of province Punjab, Pakistan is found to be among top 10th cities with worst AQI reported globally in 2019 [2]. During the year 2019, $PM_{2.5}$ concentrations in Lahore city presented the increment of this particulate concentration almost every single day of national air quality standards [3]. Inhalation of air hazardous particles has shown the challenged disorders to respiratory problems in humans for many years [4]. Fossil fuels like coal and petroleum used by industries and factories along with motor vehicles exhausts are major source of air pollution [5]. Exposure to such particulates and some potentially toxic trace elements involved systemic pulmonary inflammation and decreased pulmonary defense mechanism [6]. A study on canines were performed on blood concentrations of potentially toxic trace elements and their correlation with

blood indices and found to have positive or negative correlation between elements and blood parameters except ferric and cadmium [7].

AQI of an environment is based on presence of these particulate matters $PM_{2.5}$, PM_{10}, ozone and oxides of carbon, nitrogen and sulphur emissions in the air [8]. Particulate matters are the heterogeneous mixture of gases suspended in air in the form of droplets [9]. Sulphur dioxide is a common pollutant and its source of emission is combustion of sulphur containing fossil fuels like coal, motor vehicles and industrial products. It is found to have more concentration of sulphur dioxide in winter season than spring season in a research [10]. It is more soluble in water and is an irritant gas which can easily be breath in lungs and cause bronchial constriction of lungs in the body [11]. Oxides of nitrogen in air as pollutant particles are also being reported to have negative effects on health status of animals [12]. Oxides of carbon mostly considered as toxic molecules which are considered to be important molecules in physiology of multiple organ systems and in restoration of homeostasis in pathophysiological state [13].

A series of observational studies have revealed about the adverse effects of air pollution on humans and animals heath [14]. A study on cats living indoor with excess particulates were more suffering from the respiratory diseases than normal household with acceptable level of $PM_{2.5}$ [15]. Another study revealed the effects of inhalation of ambient air particles in dogs by inducing the myocardial ischemic conditions on excess exposure of such pollutants [16]. Another study by [17] on two different air pollutant zones of environment was performed and revealed a significant change in blood indices of Hct and Hb concentrations. A research on Eurasian tree sparrow (a human commensal species) was performed as a study model to determine toxicological effects of particulates on hematological indices and found to have adverse affects on blood cell counts [18].

Particles characteristics show diversity with regional geographical location, season and climate conditions [19]. AQI in Lahore usually becomes worsen during winter season from November to February when agricultural farmers set light to remnants of crops resulting in production of smoke that adds to the fog (smog). From March to May in spring season, weather becomes smog free up too much extent as compared to winter season. The objective of this study is to evaluate the impact of environmental pollution in association with seasonal changes on blood indices of dogs.

Materials and methods

Study area and monitoring of air quality index:

Three areas had been sampled to study the effects of air pollution on blood indices of dogs as shown in figure 1. Each area had specific concentrations of air particulates and categorized as less polluted area (Gulberg), polluted area (Town Hall) and highly polluted area (Shadman) as shown in **table** 1. For determination of AQI, reported data from Environment Protection Department, Punjab was used (www.epd.punjab.gov.pk). The blood collections were performed from three different breeds of dogs (Labrador retrievers, German shepherds and Pit bulls) of each area in winter and spring seasons. The study was approved by university ethical committee (UVAS-DR no. 1121)

Study design:

In each season, total 45 dogs were chosen from three breeds of dogs (N=5 dogs from one breed of each area). Standard protocols for animals restraining, blood collection and blood preservation were followed. We inserted the needle of syringe in cephalic vein of dogs and collected 5ml of blood in the syringe. After blood collection, we removed the pressure over the cephalic vein and put the cotton ball on venipuncture site for 30 seconds to ensure that blood had stopped completely [20]. Then blood was poured into ethylenediaminetetraacetic acid (EDTA) tube immediately to prevent clotting of blood (Thrall *et al*. 2012). The blood samples were then dispatched to laboratory for complete blood count analysis by automated hematology analyzer (Mindray BC-2800).

Hematological parameters:

Hematological parameters include hemoglobin concentration (Hb), hematocrit volume (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells count (RBC), white blood cells count (WBC), platelets, neutrophils, monocytes, eosinophils and lymphocytes.

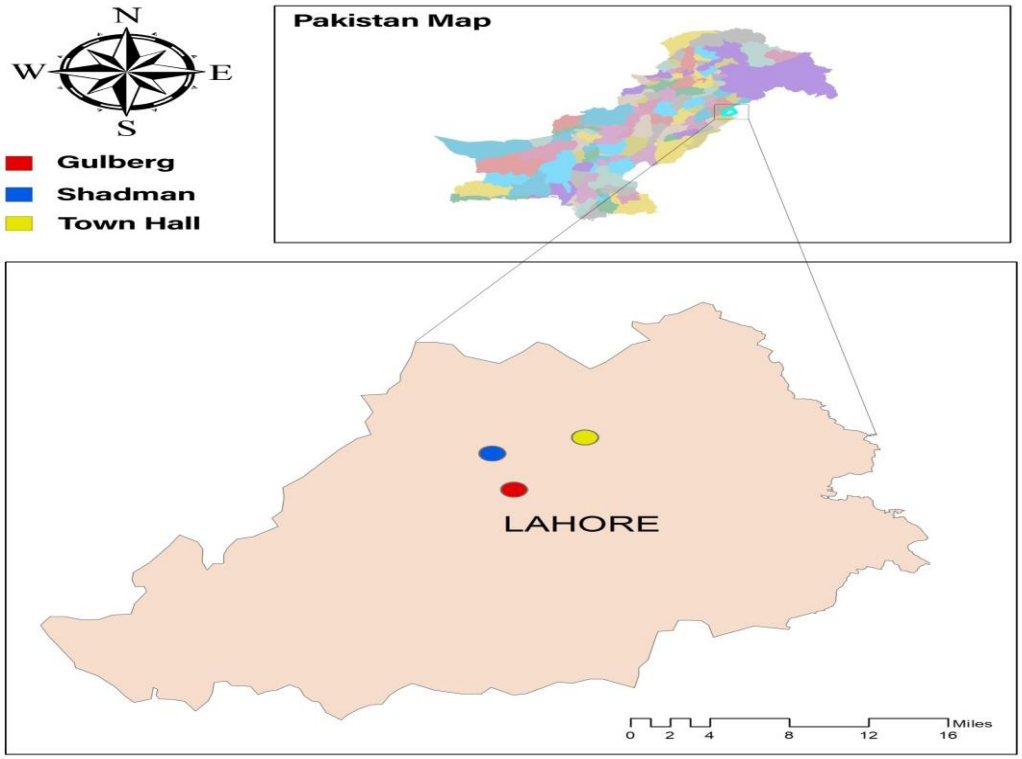

Figure 1: Map showing research designed areas of Lahore (Pakistan)

Table 1: AQI of ambient air of different areas (Gulberg, Town Hall and Shadman) of Lahore

Parameters	PM_{10} (ug/m^3)	$PM_{2.5}$ (ug/m^3)	NO (ug/m^3)	NO_2 (ug/m^3)	SO_2 (ug/m^3)	O_3 (ug/m^3)	Grade
Gulberg	82.863	71.175	SNA	SNA	42.772	17.070	Less polluted
Town Hall	SNA	SNA	18.7354	33.2066	55.826	32.2117	Polluted
Shaadman	157.027	105.447	58.184	66.153	25.258	46.686	Highly polluted

(SNA abbreviated as sensor not available)
Reported data of 06 November 2019 from Environment Protection Department of Punjab, Pakistan (www.epd.punjab.gov.pk)

***Statistical analysis*:**

The data were firstly tested for normal distribution using the Kolmogorov-Smirnov test. The data were analyzed by paired sample t-tests, followed by Tukey-post hoc tests to check the seasonal effects on blood indices of dogs using SPSS (version 20, Chicago, IL). Independent sample t-tests were performed for different areas and breeds of dogs varying in AQI to check their effects on blood profiles of dogs in winter and spring seasons. Results from hematological analysis of parameters were presented as means ± S.E.M. and p values < 0.05 were considered as significant ones.

RESULTS:

Seasonal effects of AQI on hematological indices in different areas of dogs

Using the paired sample t-tests between winter and spring seasons in different areas of dogs was analyzed. Less polluted area (Gulberg) had no significant effects on hematological indices of Labrador retrievers, German shepherds and Pit bulls in winter and spring seasons. Polluted area (Town Hall) had high significant effects on values of monocytes in Labrador retrievers, Hct and MCHC in German shepherds while decreased MCH value with significant p value in Pit bulls in winter season as compared to spring season. Highly polluted area (Shadman) had significant effects with decreased WBCs counts only in German shepherds in winter season as compared to spring season. [t] **Table** 2.

Table 2: Seasonal effects of AQI on hematological indices of dogs in polluted area

Parameters	Breeds	Mean±S.E.M (Winter)	Mean±S.E.M (Spring)
Hct (%)	Lab	40.33±3.19	48.00±3.33
	Gsd	45.56±3.21[a]	38.70±2.99[b]
	Pit bulls	40.90±0.85	45.13±3.96
MCH (pg/L)	Lab	21.40±2.28	25.16±0.98
	Gsd	22.66±0.92	21.16±2.22
	Pit bulls	23.26±0.46[a]	23.96±0.53[b]
MCHC (g/dL)	Lab	33.83±2.08	33.10±3.05
	Gsd	30.23±0.76[a]	25.96±1.79[b]
	Pit bulls	35.10±0.41	35.60±1.09
Monocytes (%)	Lab	7.70±0.65[a]	4.66±0.33[b]
	Gsd	2.33±0.33	4.00±1.15
	Pit bulls	5.66±0.33	4.66±0.88

The superscripts shown by different letters on each line are statistically different (P<0.05)
(Lab abbreviated as Labrador retrievers, Gsd as German shepherds).

Regional effects of AQI on hematological indices of dogs in winter season

Using the independent sample t-tests for regional effects on blood indices of dogs in winter season, we found Hb, RBCs counts, Hct, MCH, MCHC, monocytes and eosinophils as the significant blood parameters. Polluted area (Town Hall) had high significant level of Hb, Hct and RBCs counts than less polluted area (Gulberg) of dogs in winter season. Highly polluted area (Shadman) had high significant values of MCH, MCHC, monocytes and eosinophils than polluted area (Town Hall) of dogs in winter season. Less polluted area (Gulberg) had significant effects with decreased number of monocytes and eosinophils than highly polluted area (Shadman) of dogs in winter season.

Table 3: Regional effects of AQI on hematological indices of dogs in winter season

Parameters	Regional Areas		
	Less polluted Mean±S.E.M	**Polluted Mean±S.E.M**	**Highly polluted Mean±S.E.M**
Hb (g/dL)	16.52±0.78[a]	13.95±1.76[b]	16.21±1.15[a,b]
RBC (x10^{12} /L)	6.83±0.19[a]	6.21±0.18[b]	6.56±0.48[a,b]
Hct (%)	47.20±1.69[a]	42.26±1.56[b]	4.77±2.92[a,b]
MCH (pg/L)	24.01±0.53[a,b]	22.44±0.77[a]	24.78±0.54[b]
MCHC (g/dL)	34.80±0.85[a,b]	33.05±0.97[a]	36.04±0.46[b]
Monocytes (%)	4.11±0.97[a]	5.23±0.81[b]	8.22±1.09[c]
Eosinophils (%)	2.44±0.24[a]	3.22±0.54[b]	4.66±0.41[c]

The superscripts shown by different letters on each line are statistically different (P<0.05)

Regional effects of AQI on hematological indices of dogs in spring season

Using the independent sample t-tests for regional effects on hematological indices of dogs in spring season, we found only monocytes as the significant blood parameter in polluted area (Town Hall) and highly polluted area (Shadman) of dogs. Highly polluted area (Shadman) of dogs had high significant value of monocytes than polluted area (Town Hall) of dogs in spring season. Less polluted area (Gulberg) showed non-significant effects on blood indices of dogs in spring season.

Effects of AQI on hematological indices in different breeds of dogs in winter season

Using the independent sample t-tests in different breeds of dogs in winter season, we found only lymphocytes with significant values in Labrador retrievers and German shepherds. Labrador retrievers had high significant values of lymphocytes than German shepherds in winter season. Pit bulls showed the non significant effects on blood parameters of dogs in winter season.

Effect of AQI on hematological indices in different breeds of dogs in spring season

Using the independent sample t-tests in different breeds of dogs in spring season, we found lymphocytes with significant values only in Labrador retrievers and German shepherds. Labrador retrievers had high significant values of lymphocytes than German shepherds in spring season. Pit bulls showed the non significant effects on all blood parameters of dogs in spring season.

Discussion:

There has been an increasing trend on the significance of air particulate matter value present in our environment and its side effects on human beings and animals [21]. Many environmental departments are working on AQI like Environment Protection Department of Punjab which represents the daily AQI reports of different areas of Lahore on their official site (www.epd.edu.pk).

Seasonal study revealed that less polluted area (Gulberg) had no significant effects on hematological indices of Labrador retrievers, German shepherds and Pit bulls. A study by [22] on exposure to organic and inorganic air pollutants from automobiles also showed the limited alteration in blood indices of albino mice. Polluted area (Town Hall) had increased significant effects on values of monocytes in Labrador retrievers, Hct and MCHC in German shepherds while decreased significant effects on value of MCH in Pit bulls in winter season as compared to spring season. A past study by [23] showed the increase in values of MCHC and Hct due to air pollution in wood mouse showing the correlation with SO_2. Another study by [24] showed the elevation in monocytes associating with NO and ultrafine particles. These particulates initiate inflammatory response and involved in atherogensis by inducing oxidative stress and appear high number of monocytes as foam cells on endothelial lesion promoting the atherosclerosis [23]. High polluted area (Shaadman) had decreased significant change on WBCs count only in German shepherds in winter season as compared to spring season. Another research performed by [25] showed the negative significant relationship of air particulates matters with Hb and RBCs counts and positive significant relationship with WBCs and platelets counts in humans

Regional study in winter season showed that Polluted area (Town Hall) had decreased significant value of Hb, Hct and RBC counts than less polluted area (Town Hall) of dogs in winter season. A study on canines by (Clarke, Coull [26] showed the decrease in Hb value and is correlated with sulphur factor. The mechanistic basis and clinical impact of this observation is unclear but found change in Hb associated with elevation of particulates. A past study by (Krishnan, Sullivan [27] also showed the significant change in Hct value after exposure to diesel. Highly polluted area (Shadman) had high significant values of MCH, MCHC, monocytes and eosinophils than polluted area (Town Hall) of dogs in winter season. A past study by (Gondalia, Holliday [28] showed the higher proportion of granulocytes on exposure to air pollution. Less polluted area (Gulberg) had lower significant level of monocytes and eosinophils than highly polluted area (Shadman) of dogs in winter season. Another study on hematological profile of humans was conducted with an environment having similar climatic characteristics (altitude, temperature and humidity) but different (high and low) concentrations of ambient air pollutants. It was found with higher significant value of MCV and WBCs counts in high concentration of air pollutants but lower significant value of RBCs count, Hb, Hct and MCHC in low concentration of air pollutants of environment [29].

Regional effects in spring season were determined on hematological profile of dogs. We found only monocytes as the significant blood parameter in polluted area (Town Hall) and highly polluted area

(Shadman) of dogs in spring season. Highly polluted area (Shadman) of dogs had higher significant value of monocytes than polluted area (Town Hall) of dogs in spring season. A study on canine genes were performed to evaluate the effects of particulate matters on genomic DNA methylation and found correlated with WBC,s counts and monocytes of dogs [30]. Less polluted area (Gulberg) showed non-significant effects on hematological profile of dogs in spring season.

We found to have lymphocytes only with significant values in Labrador retrievers and German shepherds for breed wise study in winter season. Labrador retrievers had high significant values of lymphocytes than German shepherds in winter season. Pit bulls showed the non significant effects on blood parameters of dogs in winter season. Another study was performed on living being's population by (Gondalia, Holliday [31] to check effect of air pollution and was found to have significant change in values of neutrophils, white blood cells counts and lymphocytes.

We determined to have lymphocytes only with high significant values in Labrador retrievers than German shepherds for breed study in spring season. Pit bulls showed the non significant change on blood profile. Another study on air pollution and its effects on immune system was performed by [32] and found to have high lymphocytes due to air pollution and may be as proinflammatory response towards adaptive immune response mechanism.

Table 4: Seasonal and regional effects of AQI on hematological indices of dogs in winter and spring seasons

Parameters	Seasons	Less polluted Mean±S.E.M	Polluted Mean±S.E.M	High polluted Mean±S.E.M
Hb (g/dL)	**Winter**	16.52 ± 0.78^{a}	13.95 ± 1.76^{b}	$16.21\pm1.15^{a,b}$
	Spring	15.01±1.40	15.40±1.05	14.60±1.18
RBCs (x10^{12} /L)	**Winter**	6.83 ± 0.19^{a}	6.21 ± 0.18^{b}	$6.56\pm0.48^{a,b}$
	Spring	7.64±0.72	6.81±0.47	7.31±0.81
Hct (%)	**Winter**	47.20 ± 1.69^{a}	42.26 ± 1.56^{b}	$44.77\pm2.92^{a,b}$
	Spring	45.30±2.70	43.94±2.20	48.58±4.54
MCH (pg/L)	**Winter**	$24.01\pm0.53^{a,b}$	22.44 ± 0.77^{a}	24.78 ± 0.54^{b}
	Spring	23.33±1.25	23.43±0.93	21.70±1.05
MCHC (g/dL)	**Winter**	$34.80\pm0.85^{a,b}$	33.05 ± 0.97^{a}	36.04 ± 0.46^{b}
	Spring	32.84±1.88	31.55±1.79	32.10±1.43
Monocytes (%)	**Winter**	4.11 ± 0.97^{a}	5.23 ± 0.81^{b}	8.22 ± 1.09^{c}
	Spring	$4.31\pm0.74^{a,b}$	3.88 ± 0.53^{a}	5.55 ± 0.50^{b}
Eosinophil (%)	**Winter**	2.44 ± 0.24^{a}	3.22 ± 0.54^{b}	4.66 ± 0.41^{c}
	Spring	3.44±0.60	3.66±0.52	3.55±0.58

The superscripts shown by different letters on each line are statistically different ($P<0.05$).

Conclusion

Our research concluded that seasons, areas and breeds affect the blood indices with variation in air quality index. Air quality index in different areas of dogs between winter and spring seasons concluded that winter season (having poor AQI) showed the elevating level of monocytes, Hct and MCHC while depleting level of WBCs counts and MCH in dogs as compared to spring season (having good AQI).

Ethics Approval And Consent To Participate

Not applicable.

Human And Animal Rights

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

Consent For Publication
Not applicable.

Availability Of Data And Materials
None.

Funding
None.

Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

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