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High Air Co Concentration Associated with Altered RBCs Hemoglobin, and PCV: Metrologic study Sudan 2013 -2014

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ABSTRACT

Carbon monoxide is a significant air pollutant worldwide. Its poisoning causes hazards to health and damages in body. This study was conducted in Alkamleen area where a lot of red brick factories are located along the Blue Nile river bank in the east of the area. The study aimed to investigate the hematological parameter about Hb, RBC $_s$and PCV at a high CO concentration area.the study area was divided by two lines parallel to the river bank, 500 meters distance from each other into three zones A, B and C from north to south. Also it was divided from east to west by five imaginary lines to give six squires in each zone. The reading for CO concentration was determined for each square. Blood samples were collected from the habitants in the study area. Hb was determined by the Drab kin's method, Read by photoelectric colorimeter, RBCs was used improved Neubauer (chamber), to calculate the TRBC, PCV was determined by Micro-Haematocrit centrifuge. CO in air was found to be significantly higher levels of CO concentration in air were detected throughout the year in all zones. Mean+/-SD of RBCs among the study group in winter were 5.6±0.8, Mean+/-SD of RBCs among the study group in summer were 5.2±0.8. Hb, RBC$_s$, PCV were significantly higher for study group in winter as compared to control group.air CO was significantly exceeds the WHO recommended standards. Higher reading in winter and summer of Hb, RBCs and PCV were associated to high CO concentration which emitted from red brick kilns in Alkamleen area.

Introduction

The World Health Organization states that 2.4 million people died each year from causes directly attributable to air pollution. Carbon monoxide (CO) poisoning is responsible for up to 40,000 emergency department visits and 5000 to 6000 deaths per year, making it one of the leading causes of poisoning death in the United States.[i]

Carbon monoxide is a product of incomplete combustion of organic matter due to insufficient oxygen supply to enable complete oxidation to carbon dioxide (CO2). Natural background carbon monoxide concentrations average around (0.05 mg/m3) (0.04 ppm). [ii,iii]

Metrological factor effect in CO concentration in air therefore in some studies found that a concentration of three pollutants are inversely proportional to wind speed and wind direction, these factors are distributing carbon monoxide in air and can take it far away. Temperatures and humidity also effect on CO concentration.[iv]

The toxicity of CO is a result of high affinity of Hb for CO. it was found to be 200 – 250 times that for oxygen, lead to COHb formation. It also has high affinity for myoglobin, about 60 times greater than that of oxygen, which causes reduction in oxygen-carrying capacity of the blood.[v]

The absorption and elimination of carbon monoxide have been described in various mathematical models the most common of these models is the **Coburn, Forster & Kane** which takes into account all known physiological variables affecting carbon monoxide uptake.[vi]

Endogenous carbon monoxide is very small amounts formed from catabolism of hemoglobin, COHb saturation in normal physiologic level is of 0.4–0.7%, act as anti-inflammatory, vasoactive, anti-proliferative, anti-oxidant, anti-inflammatory and anti-apoptotic effects also used to prevent the development of a series of pathological conditions including ischemia reperfusion injury, transplant rejection, atherosclerosis, severe sepsis, severe malaria, or autoimmunity.[vii]

Decrease O_2 delivery to tissues leads stimulation of erythropoietin which causes increase in erythropoiesis, leading to increased red blood cell volume, Hb and Hct.[viii] Also chronic exposure can lead to appear roulex formation and black dots in blood smear. [ix]

Objective

This study aimed to investigate the correlation of high CO in air around red brick factories across seasons with changes in RBCs, Hb and PCV in Alkamleen area, central Sudan

Materials and methods

Materials

This study is a cross-sectional descriptions analytic case – control study.

Area of the study: The study was done in Alkamleen, Gazeera State, Central Sudan; it is located at the intersection of the latitude 15.23 degrees north and longitude 11.33 degrees east[x,,]. The area was mapped as follows:

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Study Area (area 1):

The area was determined from north to south along the western bank of the Blue Nile, where the redbrick kilns (source of smoke) were located. The distance was found to be three kilometers. The width was determined from east to west starting from the bank of the river and was found to be1.5 kilometer. According to carbon monoxide concentration which was high near the source, then declines while moving west till the sensitivity of the sensor reached the lowest limit and this measured was 6ppmWhich was equivalent to zero level.

Eventually this area was divided by two lines parallel to the river bank to give three horizontal zones (A,B and C) the distance between the lines was 500 meters. In east – west direction the area was divided by five imaginary lines with 500 meters apart to give six squires in each zone as seen in the diagram below.

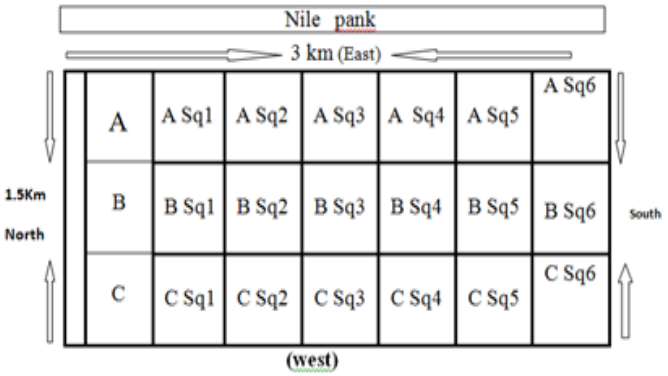

Fig 1. diagram showing the croaky of the study area into zones and squires.

Fig 2. location of the study area in Google map with a croaky of zones and squires.

Control Area (Area 2)

This area was delineated by horizontal line one kilometer west to the high way which was about three kilometers to the west of area one.

Population of the study

The study was performed on people living close to traditional kilns. Those are the families lived in this area for the last 90 years or more. It worthwhile to mention that they were living in this area before the kilns was established. The kilns were being known for the last 60 years.

Inclusion criteria

The participant were living in the study area (area one for the study group and area two for the control group) at the time of the study.

Exclusion criteria

Smokers, bakers and patients who were known cases of blood disorder. Also those who lives outside the delineated areas.

Recruitment of population

Request for participation in the study was announced to the public via the local authority.

Ethical clearance and consent

The study had been approved by the ethical committee of local committee of Alkamleen. The consent had been obtained from each volunteer after explanation of the procedure.

Methods

CO measurement in air

The tests were performed by spectrometer. The spectrometer as in fig.(3.3) was prepared and raised to height of 3 meters for 5 seconds. The reading for CO concentration, wind speed and humidity were then reported. The readings were repeated three times with an interval of 1 minute. The average reading was then determined. This step repeats five times to each square. The lower limit of sensitivity of the apparatus was 6 parts per million (ppm). This was considered as zero level

Conforms to the European Standard EN 50543
and British Standard BS 6173:2009

The high-quality testo 315-3 is an easy-to-use, ruggedly designed. CO and CO2 monitor that enables to carry out quick and reliable ambient measurements and allows temperature, humidity and wind speed measurements.

Metrological factors data

The reference for the weather climate data was obtained from Meterological Authority in Sudan2013 (see appendix).

The data included were CO concentration on air, temperature, humidity, wind direction and wind speed.

Hematological Investigations

Blood Collection and Preservation

5ml of blood were collected from peripheral vein were divided to two containers 2.5ml into heperinized container which were stored at 4c in refrigerator, and 2.5ml into EDTA container which were separated serum stored in freezer less than -20c.

Hb was determined by the Drabkin's method:

To determine the hemoglobin concentration in blood samples we used the hemoglobin cyanide technique.

Whole blood was diluted in amodifieddrabkens solution which contain potassium terricyanide and potassium cyanide. The red cells were hemolized and the hemoglobin is oxidized by the terricyanide to methaemoglobin. This was converted by cyanide to stable hemoglobin cyanide (HICN). As following steps:

1. 1.95 ml of distilled water added to 5ml of Drabkin solution to prepare it at suitable degree of concentration.
2. Mixing 0.01 ml of blood to 2ml of Drab kin solution, by shaking well the glass tube.
3. Read by photoelectric colorimeter. The colorimeter wave length was placed at 540 nm. The absorbance of the samples was read at (zero of the colorimeter).
4. By using the factor (6.8) and the concentration of the standard to have Hb concentration percentage should applied the following equation:

$$Hb = \frac{\textbf{Abs X conc (sample)}}{\textbf{Abs (standard)}} \textbf{x } 6.8$$

Measurements of PCV:

PCV was determined by Micro-Haematocrit centrifuge from (0 to 15), and Micro-Haematocrit reader from (0 to 100). As follows:

1. Blood was taken by capillary tube.
2. Then put it in Micro-haematocrit centrifuge, plasma was separated in the top.
3. Position tube in slot of Micro-Haematocrit reader line so that the base line intersects base of red cells.
4. Move tube holder so that the top line intersects top of plasma, then adjust knob so that the middle line intersects top of red cells.
5. Finally, read percentage PCV on scale.

Measurements of RBCs in blood

4 mls of 3% formal citrate (diluting fluid)wereAdded to 0.02 ml of blood. Then put one drop of mixture in Improved Neubauer (chamber), which used to calculate the result, and using x40 count the number of the cells in 80 smallest squares (ie) the 5 big squares each containing 16 small sqrs.

Calculate t using formula:

T R B C = N (80 squares) X10000 =---million\cmm

Results

Table 1. The Meteorological Data in both Areas 1 and 2 at Summer and Winter*.

No	Item	Winter	Summer
1	Wind direction	North	South west
2	Temperature	34.9 ^{0}C	43.1^{0}C
3	Humidity	29	26
4	Wind speed	5X10^6	5X10^6

**the readings were found to be similar to reports of Weather Climate Data-Meteorological Authority (see appendix).*

Table 2. Level of CO Concentration across all Zones in Winter &Summer.

Zones	CO Conc. During winter	CO conc. During Summer	S.E±	t. value	Sig.
A	104.2	76.6	14.2	2.6	*
B	54.8	48.4	8.1	0.79	Ns
C	18.9	13.9	2.5	2.04	*
Mean	59.3	46.3	7.1	1.83	Ns

ns: not significant
**: significant at 0.05 level of probability.*

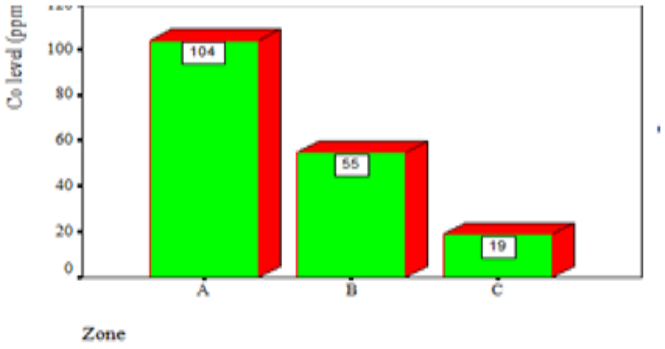

Fig 3. CO Concentration during Winter at all Zones.

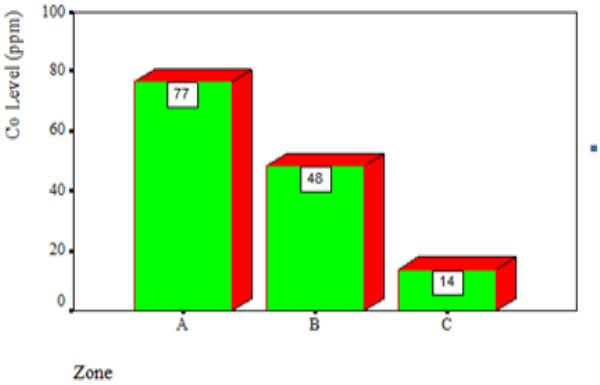

Fig 4. CO Concentration During Summer at all Zones.

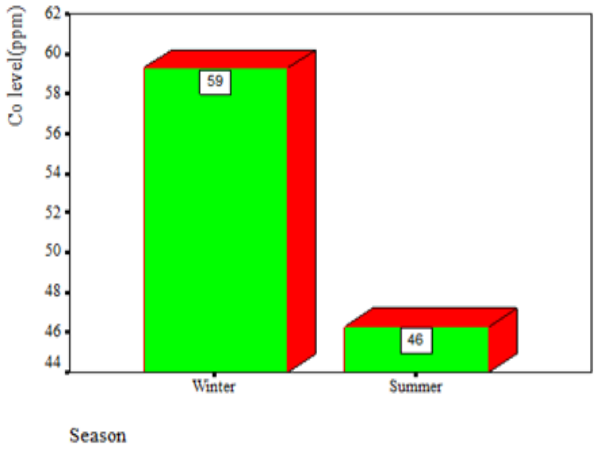

Fig 5. CO Conc. in Winter and Summer at Study Area.

Table 3. Comparison of Hematological Findings of the Study Group in Winter and Summer.

	Winter	Summer		
	Mean+/-SD	Mean+/-SD	t- value	Significance
Hb	14.4±1.9	14.6±1.7	0.73	Ns
RBCs	5.6±0.8	5.2±0.8	2.57	*
PCV	43.8±5.1	43.3±5.2	0.45	Ns

ns: not signification. *: significant at 0.05 level of probability. **: significant at 0.01 level of probability.

Table 4. Comparison of Hematologicalparameters between the Control Group and the Study Group in Winter.

Parameters	Control	Study group	t.value	Degree of Sig.
Hb	12.60	14.4	4.82	**
RBCs	4.8	5.6	6.40	**
PCV	38.4	43.3	5.16	**

ns: not signification. *: significant at 0.05 level of probability. **: significant at 0.01 level of probability.

Table 5. Comparison of Hematological Data between the Control Group and the Study Group in Summer.

Items	Control group	Study group	S.E±	t.value	Sig.
Hb	12.6	14.6	0.3	5.92	**
RBCs	4.8	5.2	.0.	3.57	**
PCV	38.4	43.3	1.0	5.16	**

ns: not signification. *: significant at 0.05 level of probability.
**: significant at 0.01 level of probability.

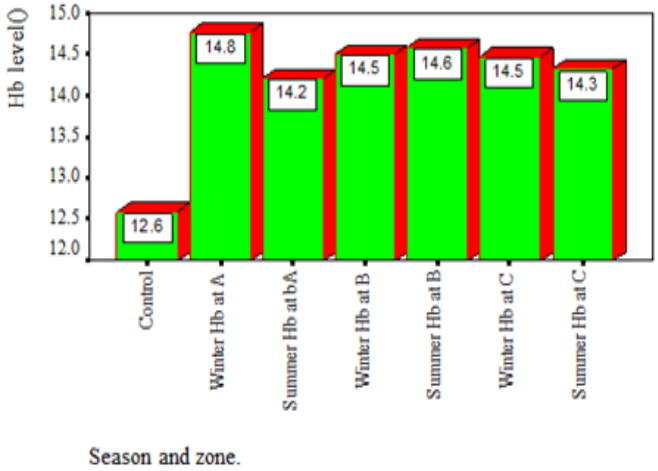

Fig 6. Comparison of Hb Concentration Between the Control Group and the Study Groups in all Zones across Seasons.

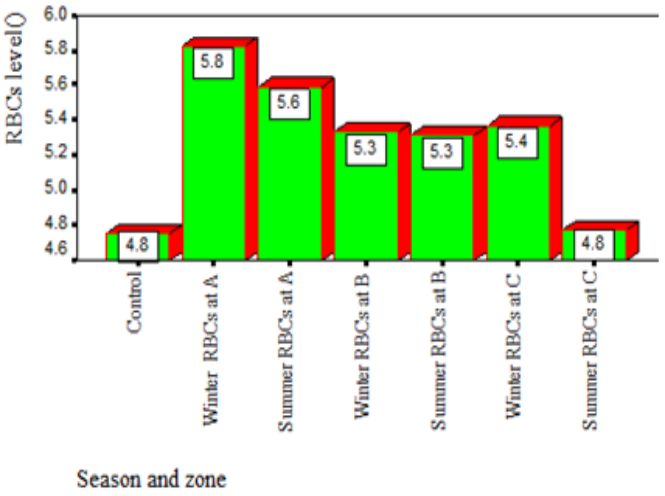

Fig 7. Comparison of RBCs between the Control Group and the Study Groups in all Zones across Seasons.

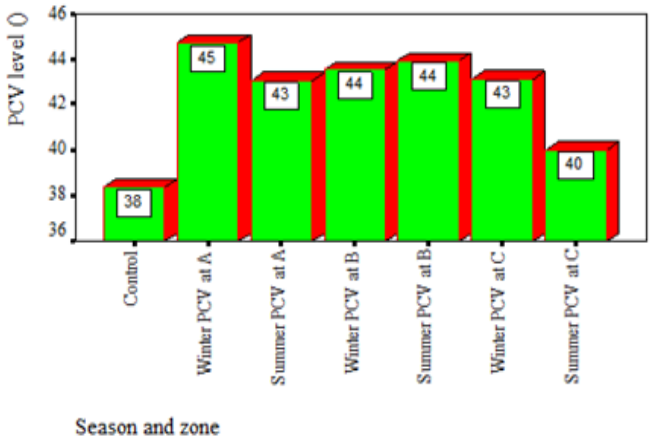

Fig 8. Comparison of PCV between the Control Group and the Study Groups in all Zones across Seasons.

Blood smear results

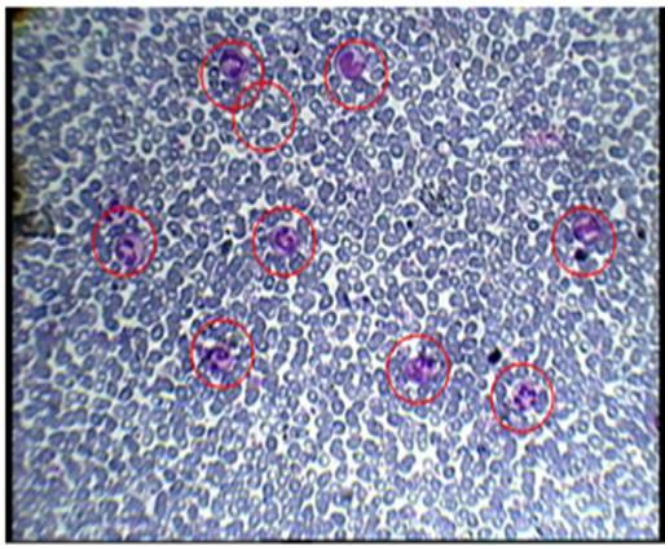

Fig 9. Peripheral Blood Film of Volunteers From The Study Area (Area 1) Showing Leukocytosis (Marked By Red Circle).

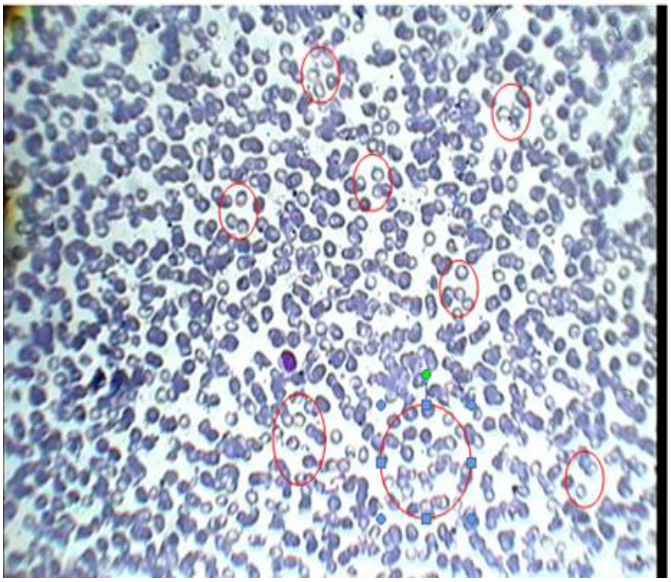

Fig 10 . Blood Film Of Volunteers In Study Area (Area 1) Showing Hypochromia.

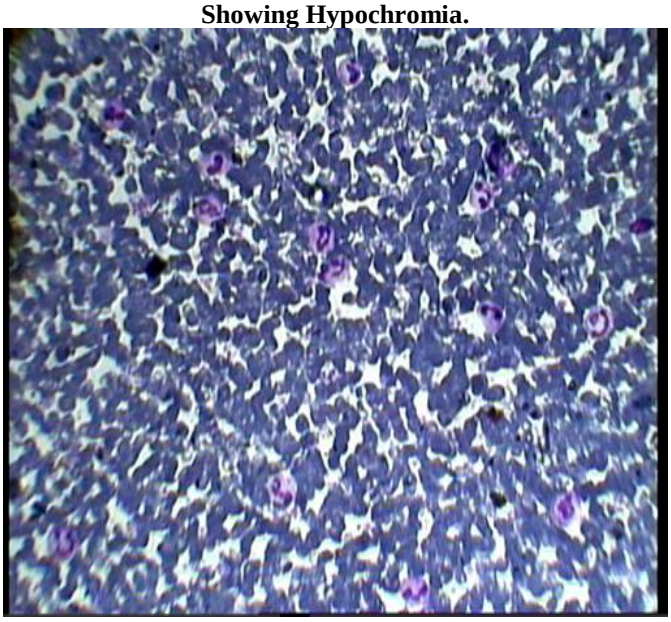

Fig 11. Blood from Volunteers In Study Area (Area 1) Showing Roloux Formation.

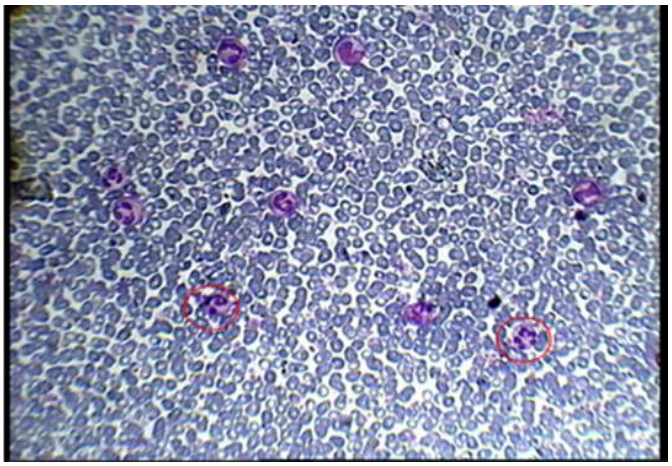

Fig 12.a and b, Blood Film From Volunteers In Study Area (Area 1) Showing Toxic Granules In The Leukocytes Which Are Pathognomonic For Chronic CO Intoxication (Demarcated By Red Circles).

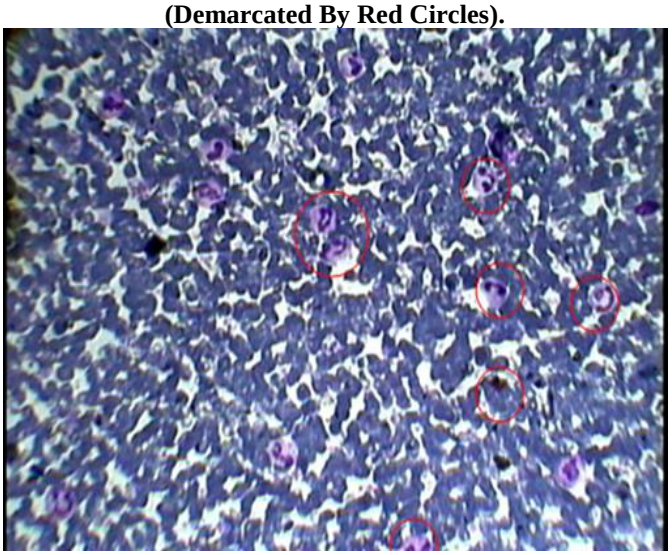

Fig 12b.

Discussion

The present study investigated the hazards related to levels of CO pollutant emitted from red-bricks kilns at Alkamleen region (North Gezira state 130 km away from Khartoum) during 2013. The site of red brick kilns is located at the east of Alkamleen extending from North to south along the western bank of the Blue Nile

*Significantly higher levels of CO concentration in air were detected throughout the year in all zones.

Wind direction had a clear effect on CO concentration in air. This was evident by detection of high level of concentration during winter in the southern squares (sq5 and 6), when the direction of wind was from north to south, while it was low during summer season when it was in the opposite direction. Another factor which affected air concentration was the distance from the source of CO the study showed higher readings in zone A compared to zone C in both winter and summer and steady readings in zone B. it worth mentioning that the levels of emitted CO violated the WHO standards during both winter and summer seasons. This agreed with results reported by researchers like Odat (2009).[xi] and Alkama*etal*, (2008) [xii] who showed that pollutants disperse away from the source of discharge till the lowest level of the sensor sensitivity (6ppm) was reached. The decrease of CO follows an exponential shape according to distances. Hence it was found beyond doubt that living near the source of CO is directly correlated to exposure to risk of high level of air pollution.

Temperature has an obvious negative correlation to CO concentration across seasons. Cold weather makes combustion less complete resulting in decreased conversion of CO to CO_2 and hence increases CO level. In contrast, higher a temperature during summer accelerates conversion of CO to Co_2 resulted in low CO levels. This finding was supported by Odat. 2009. Who showed that temperature has an inverse relation with CO level. [9]

Regarding variations in humidity, this study did not show statistically significant changes in humidity across seasons. This matched the results reported in weather-climate data 2013 from Meterological authority in Sudan. It worthwhile to mention that increased humidity leads to increase level of CO in air.[6, xiii]

The hematologic findings in general were found to be similar across all zones. This could be attributed to the fact that people do not confine to one zone i.e. they continuously have translocation movement. Another factor which could explain the similarity was that the degree of CO concentration is shown to be affected by dispersion of the pollutant resulted from changes in wind direction which may be observed within the same season. Moreover no age and gender correlation was reported with controversy. While some reporters like Sung-Soo Lee.et al.1994.[9] who conducted a study in a population similar to our work reported agreed with our results and explained it to the physiological lack of gender variation during the age of 15 to 60 years. However those who reported variations according to gender and age explained this to be due to the fact that half time of CO elimination from the blood was longer in teenagers and at lower rate than in adolescents. Also the half life of carboxyhemoglobin (COHb) during adult life was shorted in females than in males and so women were found to be more resistant to altitude hypoxia than men. [1,xiv]

Penney and Bishop (1978). found the presence of polycythemia in association with CO poisoning may be a relevant explanation to high count of RBCs in association with high CO concentration, which attributed to adaptive reaction to chronic exposure to CO.[xv, xvi] .

The finding of increased Hb and RBCs count and the PCV in the study group in summer and winter compared to the control group could be attributed to the high concentration of atmospheric CO. Chronic exposure to CO leads to reduction of oxygen delivery to the tissue causing tissue hypoxia and so reactive erythropoiesis is adequately reported in the literature.

Sung-Soo Lee.el.1994. observed similar findings of increasing number and segmentation of neutrophils in blood smear in a group of CO exposure. The absence of roulex formation in their study could be explained by the fact that they have studied the effect of acute exposure to CO in contrast to our study in which chronic exposure was the studied event Many studies observed that hemoglobin (Hb) and total RBCs count were significantly increased in workers who exposed to chronic benzene and CO levels as compared to the control group. [(xvii)].This injects in the vein of presence of polycythemia. Our finding disagreed with the study which insisted that low level of CO; did not alter Hb or Hct levels even when given chronically. [(xviii), xix]

Conclusions

The result of this study can be summarized as follows:

1. Red brick kilns are major sources of CO pollution. Concentration of carbon monoxide near the sources and surrounding zones was significantly higher depending on wind direction and intensity of emitted gases.

2. CO pollution is highly correlated to hematological changes.
3. Concentration of CO in chemical productive regions must be measured, so that protective measures should be adopted by the workers.

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