Sokoto Journal of Medical Laboratory Science 2022; 7(1): 61 - 67

SJMLS - 7(1) - 008

Effect of Soot Inhalation on Methaemoglobin and Oxyhaemoglobin Levels of some Residents of Iwofe, Port Harcourt Rivers state, Nigeria.

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Abstract

Soot is a mass of impure particles of carbon obtained from incomplete hydrocarbon combustion. Soot is an ultrafine air-borne pollutant and enters into the body through ingestion, skin contact and inhalation causing devastating effects on the blood cells. The aim of the study *was* to determine the effect of soot inhalation on methaemoglobin and oxyhaemoglobin levels of individuals resident in both Iwofe, Port Harcourt (exposed subjects) and Ihiala, Anambra state (control subjects). The study is a case control study involving residents of Iwofe, Rumuolumeni, who have been exposed to soot pollution in the environment for an average period of one year. Iwofe is in Port Harcourt, the capital of Rivers State, Nigeria. A total of fifty (50) test samples were obtained. Thirty control samples were obtained from subjects in Ihiala a city located in the South of Anambra state where illegal oil refineries and other major means of soot generation are not as comparable to what is present in Port Harcourt and its environs. Methaemoglobin and Oxyhaemoglobin concentrations were analyzed using spectrophotometric method. The data obtained was analyzed using SPSS for descriptive statistics (mean and standard deviations) and inferential statistics (t-test). The student t-test was used to test for difference in the methaemoglobin and oxyhaemoglobin levels between the exposed subjects and non-exposed controls and based on age groups and gender. An error of probability ($p \leq 0.05$) is considered statistically significant. Generally, there was a significant increase in the methaemoglobin concentrations and decrease in oxyhaemoglobin levels of exposed subjects showing the effect of soot inhalation. Activities of illegal oil refineries (a major source of soot pollution in the city) should be stopped along with other activities like burning of tyres, indiscriminate burning of wastes and gas flaring etc.

Keywords: *Soot, Methaemoglobin, Oxyhaemaglobin, Chemicals*

Introduction

Soot is a mass of impure particles of carbon obtained from incomplete hydrocarbon combustion. Soot is an ultrafine air-borne pollutant and enters into body through ingestion, skin contact and inhalation. Once inhaled, it gets into the bloodstream where it can cause devastating effects on the blood cells (their function and morphology) and the blood forming organs. For example, the chemical dust can complex with haemoglobin in the lungs to form carboxyhaemoglobin or oxidize haemoglobin to form methaemoglobin, thus impairing its function of oxygen transport. Other effects include haemolysis, platelet and white blood cell destruction (Vadivel *et al*.,2016) etc. These adverse effects of soot inhalation on the blood cells can however be determined by employing haematological assays such as the methaemoglobin estimation which determines the level of methaemoglobin in the blood.

It is no longer news that the residents in the Niger Delta part of Nigeria have lived for decades with various forms of environmental hazards that are linked to the exploitation of crude oil, a hydrocarbon (Kadafa, 2012). In recent times,

residents of Port Harcourt, River's State have recorded an upsurge in the level of soot pollution especially Obio/Akpor, Port Harcourt, Ahoada, Degema, Bonny, Okrika and Eleme Local Government Areas. This appears as fine black dusts in the air coating ground surfaces, houses, rooms, cars, clothes and any other item to which they are exposed (Victor, 2018). Soot as a byproduct of hydrocarbon combustion has been traced to high levels of emissions from expatriate companies, activities of illegal refineries in remote parts of the city which involves hacking into pipelines to steal and locally refine crude oil for sales. Those who engage in the act do not see it as theft, but as claiming what is rightfully theirs (Akintunde, 2014). To them, it is a lucrative business from which they meet their daily needs in a difficult economy and a way of getting recompense from the Nigerian state which has treated oil-producing communities like conquered territories (Sahara Reporters, 2017). Reports of a carpenter resident in Iwofe, Port Harcourt to the Punch news on May 6, 2018 attests to the fact that illegal oil refinery during nights and early hours of the morning, yields constant discharges of soot in the environment (Chukwudi, 2018). Moreover, in a quest to eradicate the illegal refineries, Federal Government agencies destroy the products by burning thereby introducing more toxic substances in the environment, thus, worsening the pollution challenge (Victor, 2018). Because of these, plumes of soot in the air have affected residents of Port Harcourt and its environs. This observation was first recorded in November, 2016 (Okumode, 2017).

Based on these, some schools of thought therefore suggest that an association exists between the emission of soot and craft-manly refining of crude oil as well as burning illegal oil refineries within the region of Port Harcourt (Kelvin,2017). Moreover, recent reports mention that law enforcement agencies do not only seize, but burn illegal refineries (Davies, 2017). According to Prof. Konya, Roseline, the then commissioner for environment, other suspected sources of soot are gas flaring, liquefied natural gas (LNG) operations and processes, petrochemical companies and refineries, burning of tires (Sweet Crude, 2017).

Several studies have not only emphasized the poor air quality in Port Harcourt, but also determined the quantity of soot (particulate matter) constituting the pollution. Sampling of two sites in Port Harcourt by the Ministry of Environment namely, Abuloma and Peter Odili Road between 12 midnight to 6am and 6am to 8am, respectively showed 270 micrograms and 62 micrograms per cubic meter of particulate matter 2.5 ($PM_{2.5}$), respectively. This exceeds the acceptable level by the World Health Organization (WHO) which is 25 microgram per cubic meter (Kelvin,2017). This proves the level of soot pollution in the city. Particulate matters of 2.5 and less are tiny enough to enter into the blood stream directly from the lungs (Jordan *et al*.,2015).

Petroleum industry constitutes a major source of air pollution in the Niger delta, especially Port Harcourt, the metropolitan and capital of Rivers state occupying approximately 1811.6km^2 area (Weli and Efe, 2015) with a population of about 1.5million (Kio-Lawson and Dekor, 2014). Within the city, petroleum industries (both international and local), production operations, gas flaring and venting as well as transportation constitute main sources of soot and other forms of air pollution besides other sources which include power plants, heavy industrial equipment and automobiles (Fagbeja *et al.,*2013). A map of Niger Delta with the ministry of environment sampling sites shows soot polluted areas. Port Harcourt is shown as an area affected by the soot pollution. The study area, Iwofe in particular is identified as an area in Port Harcourt affected by soot pollution besides other towns (Okumode, 2017). Hence, it is necessary to determine some effects of soot inhalation on the blood. This may be achieved by the measurement of haemoglobin complexes (methaemoglobin and oxyhaemoglobin) of exposed and non-exposed individuals with a comparison of any results.

Materials and Methods

Description of Area of Study

This study is a case- control study involving residents of Iwofe, Rumuolumeni, who have been exposed to soot pollution in the environment for an average period of one year. Iwofe is found in Port Harcourt, the capital of

Rivers State. It lies on a latitude of 4° 46' 38.91" North and a longitude of 7° 00' 48.24" East and the control individuals were from Ihiala a city located in the South of Anambra State where illegal oil refineries and other major means of soot generation are absent. It lies on a latitude of 4° 46' 38.91" and a longitude of 6° 51' 33.98".

Study Population

The total of fifty (50) individuals from the area made up of twenty-two (22) females and twenty-eight (28) males constituted the subjects for this study. Thirty control individuals made up of eighteen (18) females and twelve (12) males were recruited from Ihiala. Ihiala is a city located in the South of Anambra state where illegal oil refineries and other major means of soot generation are absent or less comparable to Port Harcourt. All those recruited for the study were aged sixteen to forty-five (16-45) years. Control individuals were persons who had not lived-in areas polluted with soot for a period of 1 year.

Ethical Approval

Informed consent and approval were obtained from the individuals recruited for the study.

Eligibility Criteria

All participants were apparently healthy. Pregnant females were excluded from the study. Individuals who had lived in the chosen geographical locations (Iwofe, Port Harcourt and Ihiala, Anambra State) for an average period of a year were recruited. The control subjects who had travelled to different areas of the country possibly with soot pollution and that within the past 1 year were excluded from the study. Smokers and individuals who are involved in activities that generate soot or bring them in contact with hydrocarbons were excluded from the study. Individuals who drink from water sources with uncertain purity were excluded. This is because presence of nitrates and nitrites in impure water can increase methaemoglobin levels in the blood (Manassaram *et al.*, 2010).

Sample Collection

Standard venipuncture technique was adopted for blood collection. Two milliliters of blood were withdrawn from the vein of each participant and dispensed into di-potassium ethylene diamine tetra acetic acid (EDTA) at a concentration of 0.5ml of 1.2mg/ml di-potassium EDTA. It was mixed by gentle inversion and the container labeled clearly with patient's name, gender and age. It was transported in a cold chain box with cool – packs refrigerated at 2-8°C. The samples were analyzed within 1-2 hours of collection in the Haematology laboratory, Rivers State University, Port Harcourt.

Methodology

Methaemoglobin and oxyhaemoglobin levels of the samples were determined. Evelyn and Malloy's method (Evelyn and Malloy, 1938) were employed for the measurement of methaemoglobin concentration while colorimetric method was used to estimate the oxyhaemoglobin levels.

Oxyhaemoglobin Estimation

Blood is mixed with ammoniated water which converts haemoglobin to oxyhaemoglobin. The absorbance of the solution is determined spectrophotometrically at 540nm wavelength. The concentration of the haemoglobin in the sample is directly proportional to its absorbance. Fresh ammoniated water was prepared by adding 0.04 milliliters of ammonia into 100 milliliters of distilled water in a measuring cylinder. Four milliliters of the ammoniated water were collected and dispensed into pre-labeled glass test tubes. The samples were well mixed. 0.02 milliliters of each of the well mixed whole blood samples were added into the test tubes and stoppered with rubber bungs. The absorbances of the standard and test solutions were read in at 540nm wavelength against the reagent blank. Reference Range Men: 13 – 17g/dl, Women: 12 – 16g/dl.

$$\text{Oxyhaemoglobin (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \frac{\text{Concentration of Standard}}{1}$$

Methaemoglobin Estimation Using Evelyn and Malloy's Method

Methaemoglobin has maximum absorbance at 630nm. With addition of Potassium cyanide, the absorption band disappears and the change in optical density is directly proportional to the methaemoglobin concentration. The total haemoglobin is measured after a complete

conversion to cyanmethaemoglobin by the addition of potassium ferricyanide reagent. The conversion measures methaemoglobin and haemoglobin and not sulphohaemoglobin.

A blank solution containing 4mls of solution 1 and 6mls of solution 4 was prepared and mixed in a glass test tube. 0.2mls of the sample was added to obtain D. Solution D was divided into two equal parts labeled A and B. The absorbance of A was measured at 630nm using the photo electric colorimeter to obtain D_1. A drop of solution 2 was added into D_1 and mixed. The absorbance was measured at the same wavelength (630nm) to obtain D_2. A drop of solution 3 was added to D_2 and mixed. The absorbance was measured after 5minutes to obtain D_3. A drop of solution 2 was added to B and mixed. The absorbance was measured to obtain D_4. Reference Range 1 – 2%

Note: All the measurements were made against a blank solution.

$$\text{Methaemoglobin (\%)} = \frac{D1 - D2}{D3 - D4} \times \frac{100}{1}$$

Statistical Analysis

The data obtained was analyzed using SPSS for descriptive statistics (mean and standard deviations) and inferential statistics (t-test). The sample populations were grouped into control subjects (0) and exposed subjects (1). The student T-test was used to test for difference in the methaemoglobin and oxyhaemoglobin levels between the exposed and control subjects, the age groups and gender. An error of probability ($p \leq 0.05$) is considered statistically significant.

Results

Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Exposed and Control Groups

The mean difference in methaemoglobin and oxyhaemoglobin concentrations of exposed and control groups were compared. A significant difference of p = 0.001 was observed in oxyhaemoglobin and methaemoglobin levels of the exposed and control subjects. Details are as shown in Table 1

Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Females in Exposed and Control Groups

The mean difference in methaemoglobin and oxyhaemoglobin concentrations of females in exposed and control groups were compared. A significant difference of p = 0.001 was observed in oxyhaemoglobin and methaemoglobin levels of the females in the exposed group compared with females in the control group. Details are as shown in Table 2.

Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Males in Exposed and Control Groups

The mean difference in methaemoglobin and oxyhaemoglobin concentrations of males in exposed and control groups were compared. A significant difference (p = 0.001) was observed in methaemoglobin levels of the males in the exposed group compared with males in the control group. There is no significant difference in their methaemoglobin levels. Details are as shown in Table 3.

Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations of Exposed and Control Groups Based on their Ages

Comparisons of the methaemoglobin and oxyhaemoglobin levels were made between the age groups of the exposed and control participants. There is a significant difference ($p<0.05$) in the methaemoglobin levels among the all the age groups in the exposed group compared with the control group. Only the least age group (16-20 years) has a significantly low oxyhaemoglobin level compared with other groups. Details are as shown in Table 4.

Table 1. Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Exposed and Control Groups

Variable (Years)	Exposed Group n = 50) Mean (SD)	Control Group (n = 30) Mean (SD)	p-value	Inference
Oxyhaemoglobin	11.642 (1.076)	12.333 (0.497)	0.001	Significant
Methaemoglobin	4.816 (2.436)	1.473 (0.540)	0.001	Significant

A significant difference of p= 0.001 was observed in oxyhaemoglobin and methaemoglobin levels of the exposed and control subjects.

Table 2. Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Females in Exposed and Control Groups

Variable (Years)	Exposed Female Group (n = 22) Mean (SD)	Control Fem ale Group (n = 18) Mean (SD)	P-value	Inference
Oxyhaemoglobin	10.890 (1.040)	12.174 (0.314)	0.001	Significant
Methaemoglobin	4.510 (2.462)	1.590 (0.556)	0.001	Significant

A significant difference of p= 0.001 was observed in oxyhaemoglobin and methaemoglobin levels of females in the exposed group compared with females in the control group.

Table 3. Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations between Males in Exposed and Control Groups

Variables (Years)	Exposed Male Group (n = 28) Mean (SD)	Control Male Group (n = 12) Mean (SD)	p-value	Inference
Oxyhaemoglobin	12.233 (0.660)	12.573 (0.628)	0.139	Insignificant
Methaemoglobin	5.057 (2.433)	1.298 (0.483)	0.001	Significant

A significant difference of p= 0.001 was observed in methaemoglobin levels of males in the exposed group compared with males in the control group with no significant difference in their oxyhaemoglobin levels

Table 4. Comparison of Mean Difference in Oxyhaemoglobin and Methaemoglobin Concentrations among Age Groups in Exposed and Control Groups

Variable (Years)	Age Group Mean (SD)	Exposed Group Mean (SD)	Control Group	p -value	Inference
Oxyhaemoglobin	16 – 20	11.481 (1.161)	12.438 (0.698)	0.016	Significant
	21 – 25	11.595 (1.045)	12.314 (0.298)	0.060	Insignificant
	26 – 30	11.806 (1.004)	12.336 (0.399)	0.312	Insignificant
	31 – 35	12.166 (0.931)	11.940 (0.382)	0.763	Insignificant
	36 – 40	12.405 (0.276)	12.263 (0.423)	0.711	Insignificant

Methaemoglobin	16 – 20	4.660 (2.449)	1.436 (0.559)	0.001	Significant
	21 – 25	5.113 (3.141)	1.509 (0.579)	0.003	Significant
	26 – 30	4.892 (0.937)	1.483 (0.558)	0.001	Significant
	31 – 35	4.427 (1.628)	1.057 (0.944)	0.040	Significant
	36 – 40	5.742 (2.228)	1.767 (0.611)	0.051	Significant

There is a significant difference in the methaemoglobin levels among the different age groups in the exposed group compared with the control group. Only the least age group (16-20 years) has a significantly low oxyhaemoglobin level compared with other groups.

Discussion

The levels of methaemoglobin and oxyhaemoglobin were compared between exposed and control subjects. The participants were grouped by ages and gender and their methaemoglobin and oxyhaemoglobin levels were as well compared. From the results obtained upon comparison of methaemoglobin and oxyhaemoglobin levels of exposed and control subjects, there is a significant difference in oxyhaemoglobin and methaemoglobin levels between the control and exposed groups. This agrees with their statement which says that entries of soot and petroleum products into the body have toxicological effects on the haematological system and therefore can cause many diseases in humans (Rituray and Ashwani, 2017). Also, normal methaemoglobin level is 1-2%, but with exposure to toxic chemical like exhaust fumes, dapsone, benzene, nitrobenzene etc., the methaemoglobin level increases resulting in methaemoglobinaemia (Ochei, 2008).

Subjects in the exposed group have a significantly lower oxyhaemoglobin concentration than those in the control group. The methaemoglobin concentration in the exposed group is significantly higher than those in the control group. The formation of methaemoglobin results from the reaction of haemoglobin with certain chemicals during which iron is oxidized from ferrous to ferric state which is incapable of reacting with oxygen (Ochei, 2008). Because the participants have been exposed to soot over a period of time, the inhaled pollutant (soot) oxidized haemoglobin preventing its combination with oxygen, thus, the low oxyhaemoglobin concentration. From the results obtained in the comparison of gender, there is a significant difference in oxyhaemoglobin and methaemoglobin levels between the exposed female and control female groups. Females in the exposed group have a significantly lower oxyhaemoglobin concentration and higher methaemoglobin levels than females in the control group. Males in the exposed group have significantly higher methaemoglobin concentrations than males in the control group. The grouping of the study participants by age reveals a significant difference in the methaemoglobin concentration between the exposed and control groups. Those in the exposed group have a significantly higher methaemoglobin concentration than those in the control group. Only those in the age group sixteen to twenty (16 – 20) years have a significant difference in the level of oxyhaemoglobin. The significant results in the oxyhaemoglobin and methaemoglobin concentrations of all the exposed participants prove the statement which says that all residents in Port Harcourt, irrespective of the age and gender are at risk following the inhalation (Okumode, 2017).

Conclusion

A significant difference was observed in the methaemoglobin and oxyhaemoglobin levels between the exposed subjects and the non-exposed controls. Generally, there is a significant increase in the methaemoglobin concentrations and decrease in oxyhaemoglobin levels of exposed subjects showing the effect of soot inhalation. Activities of illegal oil refineries (a major source of soot pollution in the city) should be stopped along with other activities like burning of tyres, indiscriminate burning of wastes and gas flaring etc. There is an urgent need to identify and implement alterative means for disposing petroleum products confiscated by Government agencies rather than burning. Use of face masks during visits to soot polluted areas or while engaging in activities that produce soot is advocated. Proper coverage of doors and windows of houses with the use of air conditioners to prevent the entry of soot and finally the general public should be educated on

the dangers of soot inhalation and the need for implementation of safety precautions.

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Citation: Serekara Gideon Christian, Orokwu Eziaku Chukuigwe-Igbere, Ransom Baribefii Jacob and Happiness Nkiruka Ejimmadu. Effect of Soot Inhalation on Methaemoglobin and Oxyhaemoglobin Levels of some Residents of Iwofe, Port Harcourt Rivers state, Nigeria. ***Sokoto Journal of Medical Laboratory Science; 7(1): 61 - 67.***