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Carboxyhaemoglobin, Methaemoglobin and Sulphaemoglobin Level are Increased in Automobile Workers and Spray Painters; A Study in Calabar Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OIM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ISE and TBE managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Methemoglobin (Hi) is a metaloprotein in which the iron in the haem group is in ferric state. Sulphaemoglobin (SHb) is a stable, green- pigmented molecule, which is made by the oxidation of the iron in haemoglobin to a ferric state by drugs and chemicals that contain sulphur. Carboxyhaemoglobin (COHb) is a stable complex of carbon monoxide and haemoglobin that forms in red blood cells upon contact with carbon monoxide (CO).
Aim: This study set out to determine Hi, SHb and COHb level of automobile workers and spray painters in Calabar Nigeria. Ninety subjects were recruited for this study, 30 automobile workers, 30 spray painters and 30 non-automobile workers and non-spray painters.

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Methods: Standard Evelyn and Malloy method was used for Hi and SHb estimation, Differential Spectrophotometric method was used to measure COHb while microhaematocrit method was used to determine packed cell volume.
Results: The result of this study shows that mean Hi and COHb level were significantly higher (p = 0.02) in spray painters and automobile workers than in the control. The mean Hi, COHb and SHb value of automobile workers and spray painters that are smokers were shown to be significantly higher than those that are non smokers. The result also shows that the COHb, Hi and SHb level of spray painters significantly increased as the duration of work increases.
Conclusion: This study has shown that there is increased level of Hi, COHb and SHb among automobile workers and spray painters in Calabar Cross River State, Nigeria. We therefore recommend enlightenment campaign to educate these set of workers on the negative health implication of exposure to the fumes, gasoline and spray paint.

Keywords: Methaemoglobin; sulphaemoglobin; spray painters; automobile workers; carboxyhaemoglobin.

1. INTRODUCTION

Haemoglobin is one of the two oxygen binding proteins found in vertebrates; its function is to carry oxygen in the blood from the lungs to other tissues in the body. Carboxyhaemoglobin (COHb) is a stable complex of carbon monoxide and haemoglobin (Hb) that forms in red blood cells upon contact with carbon monoxide (CO). Exposure to small concentrations of CO hinders the ability of Hb to deliver oxygen to the body, because carboxyhaemoglobin forms more readily than does oxyhaemoglobin (HbO_2). CO is produced in normal metabolism, within living organisms by natural degradation of hemoproteins, example haemoglobin, myoglobin, cytochromes [1,2] and is also a common chemical often produced in domestic or industrial settings by motor vehicles that run on gasoline, diesel, methane, or other carbon-based fuels, gas heaters and cooking equipment that are powered by carbon-based fuels such as propane, butane and charcoal [3,4]. Exposure at 100 ppm or greater can be dangerous to human health [5].

Methylene chloride ($MeCl_2$) a chlorinated hydrocarbon of relatively low flash point, widely used as solvent for Cellulose esters, fats, oils, resins and rubbers. It forms a large proportion of certain proprietary paint removers and also it is used in the paint trade to raise the flash points of lacquers [6]. When inhaled from spray paints, the liver is the primary site of metabolism where significant amount are bio-transformed to carbon monoxide (CO) [7] and the primary target organ for toxicity is the central nervous system [8,9]. Human subjects exposed to concentration of 550 ppm or less for 1 hour, have COHb levels of 1-4% and to 10% saturation within 1 hour after exposure to 1000pm for 2 hours [10]. Human exposure to Carbon monoxide and methylene chloride is mainly due to inhalation and it is usually occupational in nature, automobile workers and spray painters are particularly at risk.

Carbon monoxide is colorless, odorless, tasteless, and non-irritating gas. The exposed person is usually unaware of its effects until serious disorders occur. The poisoning by CO has become very common and may lead to significant complications such as lightheadedness, confusion, headache, vertigo, flu-like effects, and significant toxicity of the central nervous system, heart and death [11]. During daily activities, human receives low concentration doses of carbon monoxide (CO) proportional to different working environment. Some workers bear high risks for CO exposure particularly vehicle drivers, those working at garages and maintenance, traffic police men and workers at kitchens as well as car spray painters [12].

Carbon monoxido gas enters the blood system during normal breathing. Inhaled CO combines with hemoglobin to form carboxyhaemoglobin (COHb) [13]. Once this conversion occurs, the hemoglobin is no longer available for transporting oxygen to other parts of the body. As the amount of CO increases in the bloodstream, the tissues become hypoxic [14,15]. Carboxyhaemoglobin occurs in humans naturally at low concentration as an endogenous pigment derived from the breakdown of red cells [16]. The rate at which carboxyhaemoglobin accumulates in the body is a factor of the concentration of gas being inhaled (parts per million or percent) and duration of exposure. Aggravating the effects of exposure is

the long half-life of carboxyhaemoglobin in the blood stream [17]. Carbon monoxide can also occur in the presence of other toxins, complicating both diagnosis and treatment. It is a major contributor in the thousands of smoke-inhalation deaths that occur each year. People who work with spray paint, a paint stripper, can be poisoned because the fumes are readily absorbed and converted to carbon monoxide in the liver. In such cases, peak carboxyhaemoglobin (COHb) levels may be delayed and prolonged because of ongoing production [18]. In a study conducted by Banjoko et al. [13] on Methylene chloride exposure and carboxyhaemoglobin levels in cabinetmakers in different parts of Ibadan Nigeria, they showed that cabinetmakers were averagely exposed to a significantly higher carbon monoxide level compared to the control group.

Methaemoglobin contains iron in the ferric state (Fe^{3+}) rather than the reduced ferrous form (Fe^{2+}) [19]. This structural change causes an alteration of haemoglobin in the blood especially in its ability to bind to oxygen [20]. Haemoglobin when oxidised from normal divalent state to a trivalent state, the resulting brownish pigment is methaemoglobin. Sulphaemoglobin often accompanies drug-induced methaemoglobin; sulphaemoglobin can be due to exposure to substance containing sulphur atoms having the ability to bind to haemoglobin. Methaemoglobin and sulphaemoglobin cannot combine with oxygen and is associated with cyanosis (a blue discolouration of the skin due to low oxygen circulation in the blood) [21]. Haematological changes have been reported for many years due to exposure to pollutants and are being commonly used to assess human health and well-being [22]. Such health problems raise public health concerns since they affect the work force of the economy and thus putting economic constraints on the nation [23].

Previous studies have shown that exposure of automobile workers and spray painters to pollutants like automobile exhaust gas, petrol and spray paints causes oxidation of haemoglobin to methaemoglobin and sulphaemoglobin [24]. The aim of this study is to provide information on the level of carboxyhaemoglobin, methaemoglobin and sulphaemoglobin among automobile workers and spray painters in Calabar, Cross River State. Nigeria.

2. MATERIALS AND METHODS

Thirty (30) automobile workers and 30 spray painters were recruited from Calabar, Cross River state while 30 non-automobile workers and non-spray painters residing in Calabar, Cross river state, were recruited as control. They were all within the age range of 18 and 55 years. The age range was chosen because the range covers the active work force in the study area. Those workers that have not worked for six months were excluded from the study. The benefits and the importance of the study were duly explained to them in English and Efik (Local) Languages and their informed consent obtained.

Ethical clearance for this study was sought and obtained from the Cross River State Ministry of Health Ethical Committee. Blood samples were collected by venipuncture from both the subjects under study and the controls. Four milliliters (4ml) of blood was collected into EDTA (Ethylenediamine tetra - acetic acid) sample bottles and immediately tightly covered. The samples were used to estimate carboxyhaemoglobin, methhaemoglobin, sulphaemoglobin and packed cell volume within two hours of sample collection. Packed cell volume was estimated using Standard microhaematocrit method.

Methaemoglobin Estimation by Standard Evelyn and Malloy method [25].

Principle

Hi has a maximum absorption at 630 nm. When cyanide is added, this absorption band disappears and the resulting change in absorbance is directly proportional to the concentration of Hi. Total Hb in the sample is then measured after complete conversion to HiCN by the addition of ferricyanide–cyanide reagent. The conversion will measure oxyhaemoglobin and Hi but not SHb. Thus, the presence of a large amount of SHb will result in an erroneously low measurement of total Hb. Turbidity of the haemolysate can be overcome by the addition of a nonionic detergent.

Reagents

Phosphate buffer: 0.1 mol/l, pH 6.8
Potassium cyanide: 50 g/l
Potassium ferricyanide: 50 g/l
Nonionic detergent: 10 ml/l

Lyse 0.2 ml of blood in a solution containing 4 ml of buffer and 6 ml of detergent solution. Divide the lysate into two equal volumes (A and B). Measure the absorbance of A in a spectrophotometer at 630 nm (D_1). Add 1 drop of potassium cyanide solution and measure the absorbance again, after mixing (D_2). Add 1 drop of potassium ferricyanide solution to B, and after 5 min, measure the absorbance at the same wavelength (D_3). Then add 1 drop of potassium cyanide solution to B and after mixing make a final reading (D_4). All the measurements are made against a blank containing buffer and detergent in the same proportion as present in the sample.

Calculation:

Hi% = **(**D1 – D2/ D3 – D4) x (100 / 1)

Sulphaemoglobin Estimation by Standard Evelyn and Malloy Method [25,26].

An absorbance reading at 620n measures the sum of the absorbance of HbO_2 (oxyhaemoglobin) and sulphaemoglobin (SHb) in any blood sample. in contrast to HbO_2 the absorption band due to sulphaemoglobin is unchanged by the addition of cyanide, the residual absorbance as read at 620 nm is therefore proportional to the concentration of sulphaemoglobin.

The absorbance of HbO_2 alone at 620nm can only be inferred from a reading at 578nm, and a conversion factor[38], A^{578}/A^{620}, has to be determined experimentally for each instrument on a series of normal blood samples. The absorbance of sulphaemoglobin (SHb) is obtained by substance reacting the absorbance of the oxyhaemoglobin (HbO_2) from that of the total haemoglobin (Hb).

Calculation:

SHb%: $2xA^{620}$ SHb / A^{620} HbO_2

Where;

A^{620} HbO_2= Absorbance read at 578nm / Conversion factor

and A^{620} SHb = A^{620} total Hb - A^{620} HbO_2

Carboxyhaemoglobin levels in the blood samples were determined by the differential spectrophotometric, method of Van Kampen and Zijlstra [27].

Principles

Oxyhaemoglobin, but not COHb, is reduced by sodium dithionite and the percentage of COHb in a mixture can be determined by reference to a calibration graph.

Procedure for Carboxyhaemoglobin Estimation

1. 0.1 ml of blood was mixed with 20ml of 0.1% ammonia solution.
2. 20mg of sodium dithionite (Na_2 S_2 O_4) was added.
3. The absorbance was recorded at a wavelength of 538nm and 578nm (isobestic wavelength) using a spectrophotometer.
4. The measurements were carried out within 10 minutes of the addition of Na_2 S_2 O_4 .
5. The percentage COHb saturation was determined and calculated from the equation.

% COHb = 2.44.x A^{538} / A^{578} – 2.68

Data obtained were analysed using the Statistical Package for Social Sciences (SPSS version 16.0, SPSS Inc., Chicago, US).The level of significance were analysed using Independent sample t- test and one-way analysis of variance (ANOVA) and a confidence level of $p< 0.005$ was considered as significant.

3. RESULTS

Table 1 shows the mean Hi, COHb, SHb and PCV level in automobile workers and spray painters studied in Calabar, Cross River State. The mean $\pm$ SD carboxyhaemoglobin level of 1.71 $\pm$ 0.68% and 2.47 $\pm$ 1.28% were recorded for automobile workers and spray painters respectively while carboxyhaemoglobin level of control subjects was 1.18 $\pm$ 0.73%. The mean Hi value of automobile workers was 6.31±4.23 while that of spray painters was 8.60±4.23 also the mean SHb value of automobile workers was 0.41±0.36, while that of spray painters was 0.60±0.52, the mean PCV value of automobile workers was 40.7±2.95 while that of spray painters was 38.50±2.90. Statistical difference was observed in mean Hi, COHb, SHb of automobile workers and spray painters ($p < .00$) when compared to the control.

Table 2 shows the mean Hi, COHb, SHb and PCV level in automobile workers that are smokers (AM-S) and automobile workers that are non smokers(AM-NS). The mean carboxyhaemoglobin level of 1.78 ± 0.6% was recorded for automobile smokers and 1.66 ± 0.74% for automobile non-smokers with significant increase of carboxyhaemoglobin level in automobile smokers (p = .00).The mean Hi value of automobile workers that are smokers was 7.54±5.20 while that of automobile workers that are not smokers was 5.23±4.10. The mean SHb of automobile workers that smokes were 0.40±0.10 while that of those that do not smoke was 0.30±0.30, the table also showed that the mean PCV of automobile workers that smoke was 44.2±2.60 while that of automobile workers that are not smokers was 41.20±2.63. A statistical difference was observed in mean Hi, COHb and SHb of automobile workers that are also smokers when compared to automobile workers that do not smoke.

Table 3 shows the mean Hi, COHb, SHb and PCV of spray painters that are smokers (SP-S) and spray painters that are non-smokers(SP-NS). The mean carboxyhaemoglobin level of 4.4 ± 1.05% was obtained for spray painters who are also smokers and 2.08 ± 0.93% for spray painters non-smonkers with a significant increase in carboxyhaemoglobin level of spray painters who are also smokers (p = .00). The mean Hi value of spray painters that are smokers were 8.00±2.40 while that of spray painters that do not smoke was 7.66±2.74, the mean SHb value of spray painters that smokes were 0.60±0.20 while that of spray painters that do not smoke is 0.50±0.22, the mean PCV was also calculated, for spray painters that smokes the value was 45.2±4.80 while that of non-smokers was 41.8±1.64. The values were significantly higher among spray painters who are also smokers.

Table 4 shows the mean Hi, COHb, and SHb of automobile workers and spray painters based on duration of exposure respectively. Hi and COHb showed apparent increase that is not statistically significant as the duration of exposure increases (p= .47; .38) in both automobile workers and spray painters. SHb showed statistically significant increase (p= .01) as the duration of exposure increases in spray painters.

Table 1. Methaemoglobin, sulphaemoglobin, carboxyhaemoglobin and packed cell volume of automobile workers and spray painters in Calabar cross River State

Subjects(n=30)	Hi(%)	SHb(%)	COHb(%)	PCV(%)
Automobile workers	6.31±4.26*	0.41±0.36*	1.71 ± 0.68	40.7±2.95
Sray painters	8.60±4.23*	0.60±0.52*	2.42 ± 1.28*	38.50±2.90
Controls	1.38±0.80	0.18+0.42	1.18 ± 0.73	45.80±2.80
P – value	.001	.02	.02	.52

*KEY: Hi =methaemoglobin . SHb =sulphaemoglobin, COHb = carboxyhaemoglobin all in percentage. n = number of subjects; *significant when compared to that of the control*

Table 2. Methaemoglobin, sulphaemoglobin, carboxyhaemoglobin and packed cell volume of automobile workers that are smokers and non-smokers in Calabar cross River State

Subjects	Hi (%)	SHb (%)	COHb(%)	PCV (%)
AM-S(n=11)	7.54±5.20	0.40±0.10	1.78 ± 0.6*	44.2±2.60
AM-NS(n=19)	5.23±4.10	0.30±0.30	1.66 ± 0.74	41.20±2.63
p – values	.00	.01	.00	.00

KEY: Hi =methaemoglobin . SHb =sulphaemoglobin , COHb = carboxyhaemoglobin all in percentage. n = number of subjects, AM-S =automobile workers that are smokers. AM-NS = automobile workers that are non-smokers

Table 3. Methaemoglobin, sulphaemoglobin, carboxyhaemoglobin and packed cell volume of spray painters that are smokers and non-smokers

Subjects	Hi(%)	SHb(%)	COHb(%)	PCV(%)
SP-S(n=5)	8.00±2.40	0.60±0.20	4.4 ± 1.05*	45.2±4.80
SP-NS(n=25)	7.66±2.74	0.50±0.22	2.08 ± 0.93	41.8±1.64
P - values	.01	.01	.00	.00

KEY: SP-S = spray painters that are smokers. SP-NS= spray painters that are non-smokers

Table 4. Carboxyhaemoglobin, methaemoglobin and sulphaemoglobin level of automobile workers and spray painters based on duration of work exposure

	Automobile workers			Spray painters		
	COHb (%)	Hi (%)	SHb (%)	COHb (%)	Hi(%)	SHb(%)
6months -5years	1.56 ± 0.47	4.98±2.96	0.37±0.38	1.92 ± 1.0	5.92±1.20	0.30±0.15
6-10 years	1.73 ± 0.78	6.64±4.10	0.39±0.39	2.07 ± 1.27	7.60±2.02	0.62±0.16
more than 10 years	1.96 ± 0.92	7.30±5.13	0.61±0.30	2.99 ± 1.40	8.91±4.65	0.64±0.51
P -value	.46	.47	.44	.16	.38	.01

4. DISCUSSION

Exposure to carbon monoxide, benzene and methylene chloride is known to cause very harmful effects on human health [28]. Spray painters were seen in this study to have a higher value of carboxyhaemoglobin (COHb), this is no doubt due to increased background CO level coupled with exposure to methylene chloride which is component of spray paints and are bio transformed to carbon monoxide. These results were obtained previously by another study in furniture workers exposed to methylene chloride [29]. The increase in carboxyhaemoglobin level of automobile workers when compared to control in this study is still a cause for concern.

This study also showed that automobile workers and spray painters had a significant higher level of methaemoglobin and sulphaemoglobin than the controls this agrees with the report of Udonwa et al. [24] who also obtained higher level of methaemoglobin among automobile workers and petrol attendants in Calabar, this increased level may be due to constant exposure of automobile workers and spray painters to motor exhaust gas, gasoline and the spray paints used to spray cars in their work environment. The methaemoglobin and sulphaemoglobin concentration in spray painters were observed to be significantly higher than those of automobile workers, this difference may be attributed to the fact that spray painters are more exposed to these toxic ingredients since they spray without protective wears resulting in inhalation of this fumes. This agrees with the report that the practice of spray painters working without protective wears was an important source of exposure [30].

Carbon monoxide is a common pollutant present in cigarette smoke and car exhaust. In this study, carboxyhaemoglobin was found to be significantly higher in automobile smokers and spray painters who are also smokers and this agrees with the work of Van and Chertow [31] which reported that a non – smoker living and working in the country side will have a lower carboxyhaemoglobin than the cigarette – smoking city slicker who spends much of his working day sitting in a slow – moving car traffic and automobile shops. In my study, I found no rules and regulations regarding worker's safety being practiced at any visiting site; it appears reasonable that poor ventilation status at work areas seems to increase risks of exposure many times, since the release of chemicals into congested work areas seems to exceed its rate of removal from there. Methaemoglobin and sulphaemoglobin level in this study were also observed to be increased in automobile workers and spray painters who were smokers than non-smokers. It has been recorded that exposure to toxic fumes including motor exhaust gas and spray paints oxidizes the hemoglobin in red blood cell to methaemoglobin and sulphaemoglobin [24], cigarette smoke has also been reported to contain chemicals like carbon monoxide, formaldehyde, hypochlorite acid and hydrogen cyanide that are capable of oxidizing haemoglobin to methaemoglobin and sulphaemoglobin [32], therefore inhalation of smoke from cigarettes by these automobile workers and spray painters who were smokers may account for increased level of methaemoglobin and sulphaemoglobin observed in them.

In this study the haematocrit level were significantly higher in automobile smokers and spray painters' smokers which tallies with the work of Whitehead et al. [33] who also observed in their study that haematocrit was significantly increased in automobile mechanics and spray painters smoking more than five (5) cigarettes per day. Smoking and exposure to cigarette smoke are important causes of direct exposure to carbonmonoxide [34,35]. It may be an indication that smoking not only enhances exposure in occupational environments but may also have exclusive effects on haematopoiesis

[36]. The excessive carbon monoxide (CO) exposure may produce polycythemia in humans as well as in animals. The half-life of the CO in the body is 3-5 hours. In a person who smokes frequently and continuously, the carboxyhaemoglobin levels increases and produces a progressive hypoxemia and as the CO binds with haemoglobin, functional anaemia is produced. This causes the impaired oxygenation of tissues and change in haematological parameters [35].

Methaemoglobin, carboxyhaemoglobin and sulphaemoglobin level were also observed in this study to increase as duration of work increases in both automobile workers and spray painters. This report was in accordance with the work of Udonwa et al. [24]. In Nigeria automobile workers and spray painters at workshops are still largely exposed to the gasoline fumes and toxic substances in spray paints for more than a typical 40-hour work week. The American Conference of Government and Industrial Hygienist (ACGIH) and National Institute of Occupational Safety and Health (NIOSH) recommend an occupational exposure limit of 23 mg/m³ for a 10-hours work day in a 40-hours work week [37].

5. CONCLUSION

In conclusion, this study has shown that there is increased level of carboxyhaemoglobin, methaemoglobin and sulphaemoglobin among automobile workers and spray painters in Calabar Cross River State, Nigeria. We therefore recommend enlightenment campaign to educate automobile workers and spray painters on the negative health implication of exposure to the fumes, gasoline and spray paint and also mandatory use of self-protective wears by these workers should be enforced by relevant government agencies and employers of labour.

CONSENT

The benefits and the importance of the study were duly explained to them in English and Efik (Local) Languages and their written consent obtained.

ETHICAL APPROVAL

Ethical clearance for this study was sought and obtained from the Cross River State Ministry of Health Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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