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Comparison of Blood Carboxyhaemoglobin Levels among Cigarette Smokers and Petrol Station Attendants with Control Subjects in Nnewi Metropolis, Nigeria.

Okeke, Chizoba Okechukwu*, Kelechi, Maryann

Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus P.M.B. 5001 Anambra State, Nigeria.

ABSTRACT

Background: Carboxyhaemoglobin (COHb) is the product of reaction between carbon monoxide and haemoglobin which results in impaired oxygen delivery to the tissue and decreased venous oxygen content. Cigarette smokers and petrol station attendants (PSAs) are both exposed to carbon monoxide habitually and occupationally. **Aim**: This study assessed carboxyhaemoglobin levels in cigarette smokers, PSA sand control subjects in Nnewi metropolis. **Methods**: Ninety (90) subjects comprising of 30 cigarette smokers, 30 PSAs and 30 apparently healthy control subjects participated in the study. Carboxyhaemoglobin was estimated using spectrophotometric method. One-way ANOVA and Pearson correlation was applied in the statistical analysis. **Results**: The mean COHb levels of smokers (0.83 ± 0.15) and PSAs (0.49 ± 0.08) were significantly higher when compared with the mean of control subjects (0.41 ± 0.01) (P<0.001 and P=0.003 respectively). However, the mean COHb levels were significantly higher in cigarette smokers compared to PSAs (P<0.001). There was a significant positive correlation between duration of smoking and number of sticks smoked and COHb levels in smokers (P<0.001). **Conclusion**: This study has identified that smokers and PSAs are at risk of carbon monoxide poisoning. However, cigarette smokers are at greater risk which correlates with duration of smoking and quantity of sticks smoked per day.

Key words: Carboxyhaemoglobin, Cigarette, Smoking, Carbon monoxide.

***Corresponding author**: Email: oc.okeke@unizik.edu.ng +234-7033484245
ORCID: Okeke CO: 0000-0003-0269-095X
Kelechi M: 0000-0002-2549-8410

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INTRODUCTION

Carboxyhaemoglobin (COHb) is a stable product that results from the reaction between haemoglobin and carbon monoxide giving rise to high level toxicity in the body with the red blood cells losing their respiratory function (1). It is a carbon monoxide (CO) derivative of haemoglobin normally found in blood at levels of less than one percent of the total haemoglobin (2). Carbon monoxide is a tasteless, odourless, colourless and poisonous gas that displaces oxygen on haemoglobin molecule forming carboxyhaemoglobin (COHb) (3). Haemoglobin has more than 200 times the affinity for carbon monoxide than it has for oxygen, thus as the carbon monoxide (CO) levels in the blood increases, the amount of oxygen transported to the body's cell decreases. It is this oxygen deprivation that makes this condition deadly.

Conversion of haemoglobin to carboxyhaemoglobin results in carboxyhaemoglobinemia or carbon monoxide poisoning (4). Carbon monoxide poisoning may be insidious, with effect of hypoxia suddenly overwhelming an individual particularly because the gas is colourless and odourless (5). At higher levels of COHb saturation, breathing may become difficult followed by loss of consciousness, collapse, convulsion, coma and death(6). Carbon monoxide poisoning is a major public health problem and may be responsible for a significant percentage of all poisoning death.

Individuals can be exposed to carbon monoxide occupationally or via their lifestyle. Carbon monoxide is present in tobacco smoke and automobile exhaust and when it is inhaled, it binds with haemoglobin to form carboxyhaemoglobin.

Carboxyhaemoglobin impairs the transport of oxygen to tissues. The main sources of COHb are tobacco smoke and gasoline engine exhaust fumes (7). According to Beck *et al.* (8), automobile exhaust is a well-known source of toxicity and exposure to carbon monoxide. Therefore, it is important to measure the levels of COHb in cigarette smokers and PSAs because the PSAs are occupationally exposed from the exhaust fumes of vehicles that come for re-fueling while the cigarette smokers are habitually exposed to carbon monoxide from the cigarette smoke.

This study which was conducted to determine and compare the carboxyhaemoglobin levels in cigarette smokers, PSAs and control subjects in Nnewi metropolis and serves as a preliminary study on the levels of carboxyhaemoglobin in these two classes of occupationally and habitually exposed individuals. It's hoped that the data obtained would be used to create awareness among cigarette smokers and petrol station attendants about the magnitude of exposure to carbon monoxide.

MATERIALS AND METHODS

The study was carried out in Nnewi metropolis Anambra State, Nigeria. Nnewi is a town that has numerous petrol stations as well as bars, drinking joints and hotels where people drink and smoke. This study was carried out in places where smokers were easily accessible and in petrol stations.

Research design

The research is a cross sectional study that assessed the level of COHb among cigarette smokers, petrol station attendants and control subjects.

Sampling techniques

The study subjects were recruited by consecutive sampling technique.

Study population

The study population comprised adults aged between 18 to 60 years who are either cigarette smokers or petrol station attendants. Apparently healthy individuals who neither smoke nor work at petrol stations served as control subjects. The study sample was 90 subjects that comprised of 30 cigarette smokers, 30 PSAs and 30 control subjects. A questionnaire was given to each subject before sample collection to collect information on the individuals lifestyle, duration of exposure and ascertain suitability for the research.

Sample Size Determination
Sample size was calculated using G*Power software (version 3.0.10). Power analysis for a One-way ANOVA with three groups showed that a total sample size of 84 was needed to achieve a power of 90 at an alpha level of 0.05.

Ethical consideration
The ethical approval was obtained from the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Ethics Committee with reference number ERC/FHST/NAU/2018/131. Informed consent of the individual was obtained verbally.

Inclusion criteria
The study included apparently healthy smokers, PSAs and control subjects within the age range of 18-60 years.

Specimen collection
Two millilitres of venous blood were freshly collected in an EDTA container and used for the measurement of carboxyhaemoglobin levels.

Estimation of carboxyhaemoglobin (by spectrophotometric method).
Twenty-five microlitres of blood was added to 3ml of haemolyzing solution in a small test tube and mixed by inverting three times. After 5 minutes, 0.1ml of this mixture was pipetted into a cuvette containing 1.15ml of the COHb diluting solution. The cuvette was covered, mixed by inverting several times and allowed to stand at room temperature for 10 minutes. The absorbance was read at 420(A420) and 432nm(A432) against a matched cuvette containing only COHb diluting solution. The COHb was calculated using the equation;

$$\frac{1- (A_R \times F_1)}{A_R\,(F_2 - F_1) - F_3 + 1}$$

Where;
A_R is the ratio of $\frac{A_{420}}{A_{432}}$ of the sample in COHb diluting solution
The three constants, F_1, F_2 and F_3 are molar absorbtivities of reduced haemoglobin and COHb at 420 and 432nm (9,10)F_1=1.3330; F_2=0.4787; F_3=1.9939

Statistical analysis
Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. One-way ANOVA was used to compare differences among groups and post hoc comparison was done to determine differences between groups. Pearson's correlation was used to test for relationships. P<0.05 was considered statistically significant.

RESULTS
The mean COHb levels were significantly higher in cigarette smokers and PSAs compared to control groups (P<0.05). Also, cigarette smokers have a significantly higher COHb levels compared to PSAs (P<0.05)(Table 1).
There was a positive non-significant relationship between the duration of total exposure (years) and daily exposure (hours) with COHb levels among PSAs(P>0.05) (Table 2).

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There was a significant positive correlation between duration of smoking in years and quantity of sticks smoked per day with COHb levels in smokers (*P*<0.05) respectively (Table 3).

There was no significant difference in COHb levels amongdifferent age groups of PSAs and smokers (p>0.05) (Table 4). Majority of the PSAs had no symptoms (39.40%) while 18.2% had symptoms of tiredness and headache (Figure 1).

Table 1: Comparisons of COHb levels among cigarette smokers, petrol station attendants and control subjects

Groups	COHb% (MEAN ± SD)
(A) Cigarette smokers (N=30)	0.83 ± 0.15
(B) Fuel pump attendant (N=30)	0.49 ± 0.08
(C) Control subjects(N=30)	0.41 ± 0.01
F(*P*) value	107.476 (<0.001) *
A vs B: *P* value	<0.001*
A vs C: *P* value	<0.001*
B vs C: *P* value	0.003*

Keys: F(*P*) value: Comparison of parameter between smokers, petrol station attendants and control subjects.A vs B: Comparison between cigarette smokers and petrol station attendants.A vs C: Comparison between cigarette smokers and control subjects.B vs C: Comparison between petrol station attendants and control subjects.

Table 2: Relationship between duration of exposure and COHb level among petrol station attendants.

Correlation	Correlation coefficient	*P*-value
Duration of total exposure (in years) with COHb levels	0.322	0.083
Duration of daily exposure (in hours) with COHb levels	-0.012	0.950

Table 3: Relationship between duration of smoking, quantity of sticks smoked per day and COHb level among smokers

Correlation	Correlation coefficient	*P*-value
Duration of smoking (in years) with COHb level	0.607	<0.001*
Quantity of sticks smoked per day with COHb level	0.761	<0.001*

* Significant at *P*<0.05

Table 4: Comparisons of COHb level among petrol station attendants and Smokers of different age groups

	COHb% Level (Mean ± SD)	
Age groups	**Petrol station attendants**	**Smokers**
18-23 years	0.47 ± 0.04	0.87 ± 0.10
24-29	0.50 ± 0.10	0.76 ± 0.12
Above 30	0.48 ± 0.06	0.83 ± 0.14
F-value	0.427	0.963
P-value	0.657	0.394

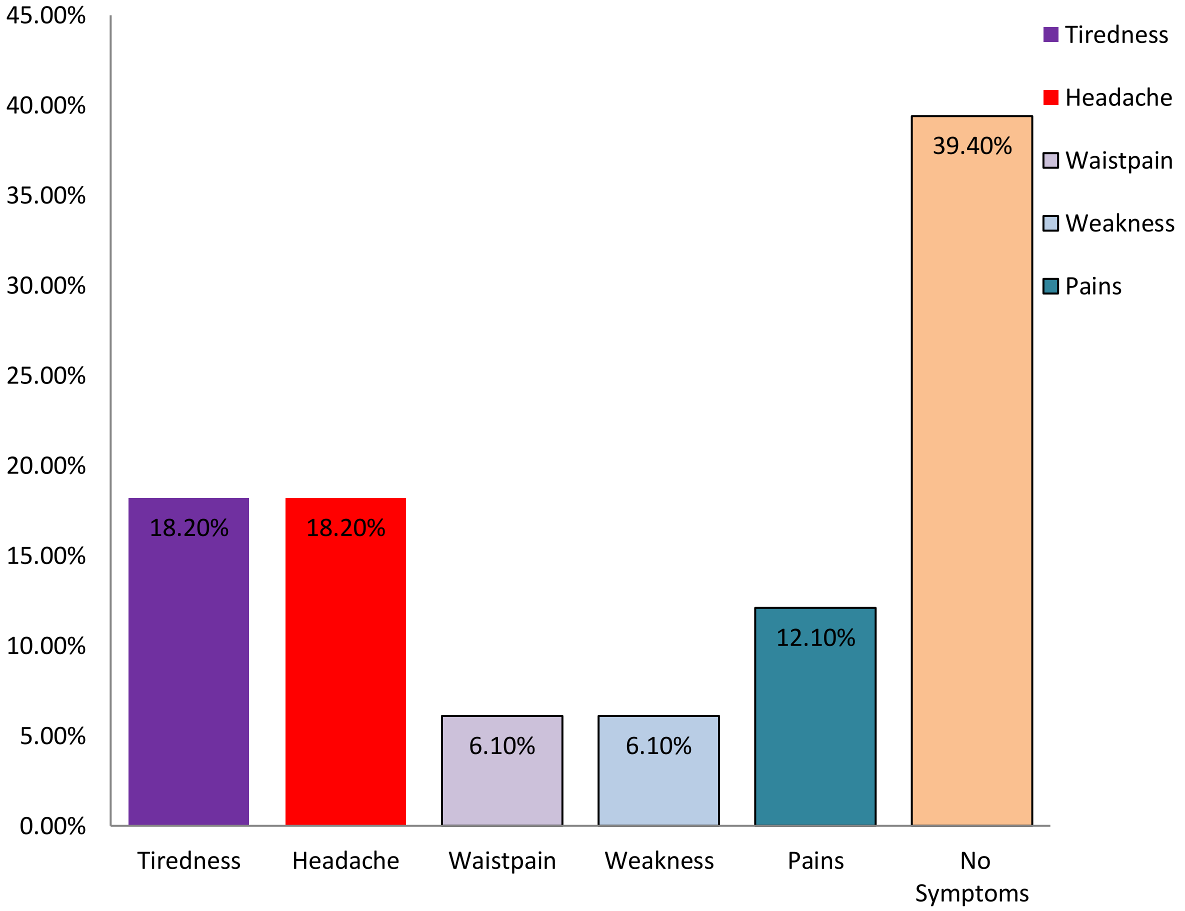

Figure 1: Bar charts showing the percentage of symptoms in petrol station attendants

DISCUSSION

This study compared the levels of carboxyhaemoglobin among cigarette smokers, petrol station attendants and control subjects. Carboxyhaemoglobin levels of the control group represent the basal level of carboxyhaemoglobin in the blood of the randomly selected unexposed individuals in the population studied. Carbon monoxide is known to be present in tobacco smoke and automobile exhaust fumes and when it is inhaled, it binds with haemoglobin to form carboxyhaemoglobin(4,11). Our finding shows that the mean carboxyhaemoglobin (COHb) levels were significantly higher in cigarette smokers compared to the control subjects (non-smokers). Carbon monoxide is one of many hazardous chemicals in cigarette smoke. There are more than 7000 chemical compounds, 250 of which are known to be poisonous andupwards of 70 that have been identified as carcinogens are present in cigarette smoke(12). Thus our finding could be explained by the fact thatthe carbon monoxide present in tobacco smoke which was inhaled by the cigarette smokers bound with their haemoglobin to form carboxyhaemoglobin. This is in line with a previous study in Lagos, Nigeria by Uko *et al.*(13) as well as the study by Cohen and Bartsch(14)and Fouad *et al*(15)that also obtained a significant higher COHb level in cigarette smokers compared to non-smokers. They agreed that smokers in the course of smoking inhales carbon monoxide (CO). Similarly, Gregory *et al.* (16) also discovered that tobacco smoking raises the blood level of COHb by a factor of several times from its normal concentrations.Several epidemiologic and pathologic studies have suggested an increased incidence of cardiovascular diseases among persons who smoke(17). The toxic effects of smoking were considered largely dependent on the nicotine content of cigarettes, but the finding of our study has highlighted that aside the long term cardiovascular effect due to nicotine, there is a short–term effect of carboxyhaemoglobinaemia which can be fatal depending on the extent and duration of exposure. According to Aronow(18), carboxyhaemoglobin levels similar to those found in heavy smokers have been reported to accelerate atherosclerotic changes and induce myocardial damage in animals, thus they considered carbon monoxide and not nicotine as the major toxic component responsible for the increase in COHb level and increased risk of cardiovascular diseases.

This study also found that the meancarboxyhaemoglobin (COHb) level was significantly higher in petrol station attendants when compared to control subjects. This can be explained by the presence of carbon monoxide in automobile exhaust fumes which these petrol station attendants are exposed to on daily basis. Petrol engines produce up to 5-7% of carbon monoxide in their exhaust fumes and more if the engine is idling, defective or improperly tuned(19). In most developing countries, pump attendants work more than eight hours per day and this has resulted to more exposure(20). Occupational exposures to automobile exhaust and petroleum fumes through inhalation of exhaust fumes and vapours during refuelling as well as other environmental hazards have been reported to have toxic effects on various body organ and systems (21).

We also found that cigarette smokers comparatively had a significantly higher mean COHb levels when compared to petrol station attendants.While the level of COHb was 19.5% higher in petrol station attendantsthan control subjects, it was over 100% higher in smokers than in control subjects. Interestingly, the level of COHb is 69% higher in smokers than petrol station attendants. This implies that cigarette smokers are more exposed to carbon monoxide and thus at higher risk of carboxyhaemoglobinaemia than petrol station attendants. This difference in carboxyhaemoglobin levels could be explained by the fact that petrol station attendants are occupationally exposed and are passive inhalers of the carbon monoxide while cigarette smokers are habitually exposed and active inhalers of carbon monoxide.

This study found a positive but non-significant relationship between the duration of total exposure (with respect to years of working) and daily exposure (based on daily hours of working) with COHb levels among petrol station attendants. This suggests that the COHblevel increases with the duration of exposure. The fact that this correlation is not significant may be explained by the fact that majority of the test subjects have worked for a limited duration (less than three years). It could also belinked to the fact that the petrol station attendants do not work in an enclosed space, thus the extent of exposure may have been diluted by the open nature of the petrol stations. According to Garland and Pearce (22), when the exhaust fumes are confined to a small place, then a dangerous or lethal level of COHb can build up in a short space of time. This may be a pointer to the extent of danger petrol station attendants could be exposed to when the petrol stations are located in a poorly ventilated environment.

The significant positive correlation between duration of smoking in years and quantity of sticks smoked per day with COHb levels in smokers in this study implies that the longer the length of time one has been smoking and the more the number of cigarette sticks smoked, the higher the level of carboxyhaemoglobin. This aligns with the finding of Nordenberg *et al*(23) that carboxyhaemoglobin levels in smokers increased progressively with the number of cigarettes consumed per day and cigarette smoking seems to have a generalized upward shift of haemoglobin distribution curve.

This study found no significant difference in COHb levels amongst different age groups of smokers and petrol station attendants. This means that age differences did not influence the levels of COHb in smokers and petrol station attendants.

Our findings shows that majority of the fuel pump attendants had no symptoms (39.40%) while 18.2% had symptoms of tiredness and headache, while only 6.1 had waist pain and weakness. According to Ernst and Zibrak(24) and Raub*et al.* (25), acute exposure to high levels of carbon monoxide produces symptoms of central nervous system toxicity. These symptoms includes; dizziness, headache, drowsiness, weakness, vomiting, nausea, irritability, confusion, disorientation, convulsions and coma. However, symptoms vary based on level of exposure. Headaches and dizziness are the most commonly reported symptoms(24). Following acute onset, delayed development of Neuro-psychiatric impairment may occur within 1-4 weeks of exposure, with symptoms including impaired judgement, poor concentration, memory loss, cognitive, and personality changes and psychosis and tissue hypoxia secondary to carboxyhaemoglobin formation may be a contributing factor(24,26).

Conclusion

Smokers and fuel pump attendants are at significant risk of carbon monoxide poisoning. There is a positive correlation with duration of smoking and number of sticks smoked per day. Since increased exposure to carbon monoxide is toxic, unhealthy and negatively affects the health condition of people who are exposed, cigarette smoking should be discouraged while petrol station attendants should be advised to wear protective masks to minimize exposure and inhalation of carbon monoxide. This will serve as a precautionary measure to protect these people from the hazards of carbon monoxide poisoning.

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