Comparative Studies of the Effects of Smoking on Haemoglobin and Haematocrit Levels between Smokers and Non-Smokers in Benin City, Edo State, Nigeria

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ABSTRACT

Background: The exact mechanisms of action of smoking in the pathophysiology of these diseases are not known but may be attributed to the abnormalities in blood rheology. **Objectives:** The aim of this study is to compare the effect of smoking on carboxyhaemoglobin (COHb), haemoglobin (Hb) and hematocrit (Hct) levels between smokers and non-smokers in Benin City, Edo State, Nigeria. **Materials and Methods**: Seventy-eight (39 smokers and 39 non-smokers) individuals participated in this study. A structured questionnaire was administered to each individual participant to elicit details such as their personal data, age, sex, residence, marital status, alcohol consumption, smoking, number sticks smoke per day, duration of smoking, as well as history of any underlying disease. Blood samples were collected from the participants and analyzed using standard methods. **Results:** This study revealed that non-smokers had a significant ($p<0.05$) higher mean haematocrit, and haemoglobin concentration than smokers, while smokers had significant higher ($p<0.05$) carboxyhemoglobin than non-smokers when they were compared. There were no significant differences in mean Hb concentration and Hct when compared based on smoking durations, current smoking status, smoking intensity, age of smokers, smokers' drinking habit and family history of smoking. **Conclusion:** The present study indicated significantly higher mean COHb, but lower Hb and Hct levels among current smokers compared with non-smoking control. Furthermore, Hb and Hct levels were not affected by the duration of smoking, current smoking status of smokers, intensity of smoking, age of smokers, alcohol habits of smokers and family history of smoking.

Keywords: Smoking, carboxyhemoglobin, hemoglobin, hematocrit

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INTRODUCTION

In 2009, the World Health Organization (WHO) reported that tobacco-related deaths would amount to 6.4 million in 2015, 8.3 million in 2030 and one billion deaths during the 21st century [1, 2, 3]. People consume tobacco in various forms which include smoking, chewing, snuffing or dipping. Data from the WHO has also revealed in 2008 that, approximately 5 million people die worldwide every year as a result of the diseases caused by smoking, and if the trend continues unchecked, it was expected that by 2015, that number would increase to 10 million. Numerous studies have indicated that smoking has adverse effects on human health, which serves as a precursor to the development of many pathological conditions and diseases, such as chronic obstructive pulmonary disease [4], cancer [5], pancreatitis [6], gastrointestinal disorders [7], periodontal disease [8], metabolic syndrome [9], and some autoimmune diseases [10]. Other studies have also shown that cigarette smoking is associated with an increased risk of cardiovascular diseases, including coronary artery disease, peripheral vascular disease (11), ischemic heart disease [12], atherosclerosis [13], myocardial infarction [13] and stroke [14]. Other noticeable and documented health problems seen in human body due to smoking include, hematological and physiological changes [15, 16]. The exact mechanisms of action of smoking in the pathophysiology of these diseases are not known but may be attributed to the abnormalities in blood rheology, infection and inflammation, oxidative stress, alteration of antithrombosis and fibrinolysis.

Smoking has been shown to cause increase in haemoglobin concentration [17], and this increase is believed to be caused by carbon monoxide exposure. The increment, especially in the mean haemoglobin levels and the carboxyhaemoglobin levels, is progressive with the number of cigarettes which are consumed per day. Carbon monoxide combines with haemoglobin to form carboxyhaemoglobin which is an inactive form of haemoglobin that has no oxygen carrying capacity. This newly formed carboxyhaemoglobin then shifts haemoglobin dissociation curve to the left thus reducing the ability of the haemoglobin to deliver oxygen to the tissues. To compensate for this low oxygen delivery capacity, smokers tend to maintain higher haemoglobin level than non-smokers [18]. In another study however, no significant difference was found in haemoglobin level between ex-smokers and non- smokers [19].

Effects of smoking on alterations of haemostatic and hematologic parameters have been extensively studied, but the studies presented inconsistent results. To the best of our knowledge, there is no known documented comparative study of the trio of carboxyhemoglobin, hemoglobin and hematocrit between smokers and non-smokers in Benin City in particular and Nigeria in general, hence the justification for this study. The present study is therefore aimed at comparing the effects of cigarette smoking, firstly on carboxyhaemoglobin (COHb), then haemoglobin (Hb) and hematocrit (Hct) levels between smokers and non-smokers in Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Study Location

This study was conducted in Benin City, Edo state, Nigeria. Benin City is a commercial center and capital of the state. Edo state is a civil service state with larger percentage of the population gainfully employed with white collar jobs. However, many others are engaged in trading, bronze casting, commercial bus driving, cart pushing, metal scavenging and several menial jobs. Most people who are engaged in jobs such as commercial bus and heavy lorry driving, truck and barrow pushing and

metal scavenging are involved in cigarette smoking. They believe it provides them with needed energy and motivation for the hard work they engage in, while to some, it is habitual. More so, the fact that there is no enforcement of 'no public smoking' law in Edo state makes cigarette smoking go on unabated.

Study Design

The study was a comparative cross-sectional study in which purposive sampling technique was done. A total of seventy-eight (39 current smokers; 39 non-smokers) males, within the ages of 15-60 years participated in this study. The smokers were selected from various motor parks and dumping sites in Benin City. The control group comprises of healthy adults of same age range, who are neither smokers nor live very close to smokers. Former smokers, female smokers as well as participants who recently lost blood, or taking medications affecting blood parameters and those who had underlying diseases, were excluded from this study. The personal consent of individual participant was sought after explaining the purpose of the research. A structured questionnaire was administered to every participant of this study.

Questionnaire / Ethical Approval

The questionnaire consisted of questions designed to elicit details about their personal data including age, state of origin, current medications, alcohol consumption, smoking habit (current, former, passive, and none), current smoking status, number of cigarette sticks smoked per day, duration of smoking, living or working in the near smokers, frequency of cigarette smoke exposure, knowledge of dangers of smoking, family history of smoking as well as history of cough and any underlying disease. The Ethical committee of the Ministry of Health, Edo State and leaders of the various motor parks and dumping sites approved this study.

Blood Collection and Analysis

Ten milliliter blood sample was collected under aseptic conditions and five milliliters was dispensed into a plain container. The non-anticoagulated blood was allowed to clot, spun at 1500 rpm for 10 minutes and the supernatant serum was separated into a separate sterile plain tube. The serum was stored at -20^{0}C for up to 2 weeks prior to analysis. The remaining five milliliters was dispensed into ethylenediaminetetraacetic acid (EDTA) container for the analysis of hemoglobin and hematocrit.

Determination of Packed Cell Volume (Haematocrit)

Packed cell volume was determined using microhaematocrit method. The determination of the packed cell volume (PCV) using a small quantity of whole blood, a capillary tube, and a high-speed centrifuge. The measure of the ratio of the volume occupied by the red blood cells to the volume of whole blood was then read with haematocrit reader and expressed as a fraction.

Analysis of Haemoglobin

Hemoglobin concentration was measured by cyanmethemoglobin method under standard protocol [20].

Analysis of Carboxyhaemoglobin

The blood concentration of carboxyhemoglobin was determined using commercially purchased ELISA kit from Calibiotech U.S.A. according to the manufacturer's instructions.

Data Analysis

Data were analyzed using SPSS version 25 (IBM, USA, 2018). Data obtained were expressed as mean and standard deviation. Comparative analysis was done using Independent sample t-test, while correlation tests were done using the Spearman correlation test. Data were considered significant at $p<0.05$.

RESULTS

Table 1 shows the demographic characteristics of the participants. Data indicates that the non-smokers

(43.58 ± 15.27 years) were significantly older ($p < 0.001$) compared with the smokers (27.87 ± 4.76 years). Majority of the participants (non-smokers, 84.6%; smokers, 79.5%) were indigenes of Edo State. Majority of the smokers (79.5%) were single, while most of the non-smokers (71.8%) were married.

Table 2 shows the smoking characteristics and alcohol habit of participants. Data indicated that the mean and SD of smoking duration among the smokers was 3.74 ± 2.24 years, while 2.05 ± 1.12 stick of cigarettes were consumed by the smokers per day. A greater percentage of the smokers (69.2%) had smoked for <5 years, while 38.5% had smoked for =5 years. Most of the smokers reported that they smoke occasionally (59%), while 41% said they smoke daily. Majority of the smokers reported they take =2 sticks of cigarette per day, while 38.5% said they consume less than 2 sticks per day. Most of the smokers (82.1%) were engaged in the habit of alcohol consumption, while most of the non-smokers (59%) do not take alcohol. All the smokers reported they were always exposed to cigarette smoke, while majority of the non-smokers reported that they are exposed to cigarette smoke once in a while. Majority of both smokers (61.5%) and non-smokers (74.4%) reported that they live and work near smokers. Most of the participants (smokers, 100%; non-smokers, 86.8%) were aware of the dangers of cigarette smoking to health. A greater percentage of the smokers (69.2%) had a family history of cigarette smoking, while most of the non-smoker (68.4%) indicated they had no family history of smoking.

Table 3 shows a comparative analysis of the mean carboxyhemoglobin, hemoglobin concentration and hematocrit between the smokers and their non-smoking controls. Independent sample t-test indicated significantly greater ($p < 0.001$) mean carboxyhaemoglobin among smokers (161.51 89.65 ng/ml) compared with the non-smokers (68.04 19.89 ng/ml). On the other hand, our data showed that the non-smokers indicated significantly greater ($p < 0.01$) mean haemoglobin concentration (13.89 0.77 vs. 13.29 1.05 g/dl) and haematocrit (41.65 2.25% vs. 39.84 3.24%) compared with the smokers.

A bivariate correlation test between the mean blood levels of carboxyhaemoglobin, haemoglobin and haematocrit in the study population is shown in Table 4. Data shows that there were no significant correlations between COHb and Hb (non-smokers, $p = 0.192$; smokers, $p = 0.379$) and Hct (non-smokers, $p = 0.315$; smokers, $p = 0.285$). In contrast, there were significant positive correlations between Hb and Hct in both non-smokers ($p < 0.001$) and smokers ($p < 0.001$).

Table 5 shows the mean haemoglobin and haematocrit levels compared according to some of the characteristics of the current smokers. Independent sample t-test indicated no significant differences in mean Hb concentration and Hct when compared between smoking durations of <5 years and =5 years; between those who smoke daily and those who smoke occasionally; between those who smoke <2 sticks of cigarette/day and those who smoke =2 sticks/day; between older smokers (=25 years) vs. younger smokers (<25 years); between those with family history of smoking and those without. Furthermore, mean HB and Hct did not also differ between smokers who drink alcohol and those who do not.

DISCUSSION

The principal findings of the present study revealed that the smokers indicated significantly higher mean COHb, but lower Hb and Hct levels compared with the non-smoking control. In addition, Hb and Hct were not affected by the duration of smoking habit, current smoking status, intensity of smoking, age of smokers, alcohol habits of smokers and family history of smoking among the current smokers respectively.

The result of our study which showed higher COHb

Table 1. Demographic Variables of Participants

Variables	Non smokers	Smokers
Age (years); mean ± SD	43.69 ± 15.27	27.87 ± 4.76*
State of origin; n (%)		
Edo State	33 (84.6)	31 (79.5)
Delta State	4 (10.3)	5 (12.8)
Ondo State	1 (2.6)	1 (2.6)
Imo State	1 (2.6)	2 (5.1)
Marital Status; n (%)		
Single	11 (28.2)	31 (79.5)
Married	28 (71.8)	8 (20.5)

Table 2. Smoking Characteristics and Alcohol Habit of Participants

Variables	Mean ± SD (Range) or n (%)	
	Non-smokers, n = 39	Smokers, n = 39
Duration of Smoking (years)	-	3.74 ± 2.24 (Range, 1 - 12)
<5 years	-	27 (69.2)
=5 years	-	12 (30.8)
Current Smoking Status		
Daily Smokers	-	16 (41.0)
Occasional Smokers	-	23 (59.0)
Smoking Intensity (sticks/day)	-	2.05 ± 1.12 (Range, 1 - 5)
<2 sticks per day	-	15 (38.5)
=2 sticks per day	-	24 (61.5)
Frequency of Exposure to Cigarette Smoke		
Once in a while	33 (84.6)	-
Every time	4 (10.3)	39 (100)
Not at all	2 (5.1)	-
Live or Work Near Smokers		
Yes	29 (74.4)	24 (61.5)
No	10 (25.7)	15 (38.5)
Knowledge of Dangers of Smoking	33 (86.8)	39 (100)
Yes	6 (13.2)	-
No		
Family History of Smoking		
Yes	12 (31.6)	27 (69.2)
No	27 (68.4)	12 (30.8)
Alcohol Intake		
Yes	16 (41)	32 (82.1)
No	23 (59)	7 (17.9)

Table 3. Comparison of The Blood Parameters Between Non-smokers and Smokers

Variables	Group	Mean	? SD	P-value	T-Statistics
Haematocrit (%)	Non-smokers	41.65	? 2.25	0.006	2.84
	Smokers	39.84	? 3.24		
Haemoglobin Conc. (g/dl)	Non-smokers	13.89	? 0.77	0.006	2.86
	Smokers	13.29	? 1.05		
Carboxyhaemoglobin (ng/ml)	Non-smokers	68.04	? 19.89	<0.001	-6.35
	Smokers	161.51	? 89.65		

Table 4. **Bivariate Correlations between the Mean B lood Levels of Carboxyh aEmoglobin, Hæmoglobin and H aematocrit in the Study population**

	Non-Smokers			Smokers		
Variables	COHb	Hb r (*p - value*)	Hct r (*p - value*)	COHb	Hb r (*p - value*)	Hct r (*p - value*)
COHb	1	0.216 (*0.192*)	0.167 (*0.315*)	1	0.145 (*0.379*)	0.175 (*0.285*)
Hb		1	0.981 (*<0.001*)		1	0.972 (*<0.001*)

Abbreviations: COHb, Carboxyhaemoglobin; Hb, Hemoglobin; Hct, Haematocrit; r, Correlation coefficient

Table 5. The Mean Haemoglobin and Haematocrit Levels Compared according to Some of the Characteristics of the Current Smokers

Characteristics	Number	Haemoglobin Concentration (g/dl)	Hematocrit (%)
Duration of Smoking			
<5 years	27	13.16 ± 1.0	39.40 ± 3.06
=5 years	12	13.58 ± 1.15	40.83 ± 3.56
		P = 0.256	*P = 0.210*
Current Smoking Status			
Daily Smokers	16	12.96 ± 1.24	38.87 ± 3.87
Occasional Smokers	23	13.52 ± 0.85	40.52 ± 2.60
		P = 0.104	*P = 0.121*
Smoking Intensity			
<2 sticks/day	15	13.55 ± 0.86	40.53 ± 2.66
=2 sticks/day	24	13.12 ± 1.14	39.41 ± 3.54
		P = 0.227	*P = 0.303*
Age of Smokers			
<25 years	9	13.30 ± 0.89	40.22 ± 2.68
=25 years	30	12.29 ± 1.11	38.73 ± 3.43
		P = 0.981	*P = 0.698*
Alcohol Habits of Smokers			
Drinkers	32	13.35 ± 1.07	40.09 ± 3.28
Non-Drinkers	7	13.01 ± 1.01	38.71 ± 3.04
		P = 0.449	*P = 0.315*
Family History of Smoking			
No	12	13.39 ± 0.55	40.33 ± 1.87
Yes	27	13.24 ± 1.22	39.62 ± 3.71
		P = 0.701	*P = 0.540*

levels among smokers compared with non-smokers is in agreement with previous studies [21, 22, 23], which have demonstrated higher levels of COHb in smokers compared with non-smokers. It is believed that the higher level of COHb among cigarette smokers could be due to greater exposure to carbon monoxide gas (CO), which is one of the inhaled components of cigarette smoke. Carbon-monoxide present in cigarette smoke is more than 600 times the concentration considered safe in industrial plants. The lower mean Hb observed in smokers is in agreement with previous studies [24, 25, 26, 27] which have demonstrated lower mean Hb levels in smokers compared to non-smokers. However, this finding is in disagreement with previous studies [28, 29, 30, 31], which found higher levels of Hb in smokers than in non-smokers. Concerning Hct, the lower mean values observed among smokers compared with non-smokers did not concur with previous studies [28, 31, 32, 33], which has shown significantly higher mean Hct levels in cigarette smokers compared with non-smokers. It is noteworthy that all the studies that indicated higher Hb and Hct in smokers than non-smokers, suggested that the increase in blood level of these parameters in smokers could be a compensatory mechanism attributable to increased hypoxia and erythropoietin-stimulating influence of smoking-induced increase in carbon monoxide. It is therefore surprising that the values of Hb and Hct in the present study were lower in smokers exposed to CO compared with the less exposed non-smokers. The reasons behind these findings are also not very clear, especially when our study indicated significantly higher COHb levels in the smokers compared with non-smokers.

The contradictory results could possibly be attributed to the fact that majority of the smokers had smoked for a shorter period of less than 5 years (n = 27; 69.2%); a greater percentage, 38.5% (n = 15) smoked one stick of cigarette per day compared to percentages of those who smoked larger number of cigarettes (e.g. 5 sticks of cigarette, 2.6%; 4 sticks, 12.8%; 3 sticks, 10.3%; 2 sticks, 35.9%) per day. In addition, majority of the smokers (59%, n = 23) smoked occasionally, while 41% smoked regularly. The lower smoking duration, lower smoking intensity and frequency as well as less sample size in the present study may have resulted in the little or no effect of CO observed on Hb and Hct levels unlike previous larger sample size studies in which subjects have smoked more regularly for longer durations and consumed larger number of cigarettes sticks per day. Interestingly too, our result indicated no significant correlations between COHb and Hb or Hct among the study population, thus suggesting lack of COHb effect on Hb and Hct.

Furthermore, it is thought that the lower mean Hb and Hct observed in the smokers in our study may be due to an interplay of other cigarette smoke-induced factors, which may have overridden the effect of CO and resulted in lower Hb values compared with the non-smokers. For example, nicotine has been shown to inhibit iron uptake, probably by acting as a weak base inhibiting iron release from transferrin [34]. At the same time, the serum levels of ferritin have been found to be low in smokers due to smoking-induced hypoxia [35]. These may explain the lower Hb level observed in smokers since iron plays a vital role as a catalyst in the synthesis of hemoglobin. In addition, it is reported that cigarette smoking decreases the levels of Vitamin C [36, 37, 38], which, in turn, predisposes the smoker to iron deficiency due to decrease in the absorption of iron. Smoking is also known to cause macrocytosis mainly by altering the levels of Vitamin B12 and folic acid [39], hence adversely affecting the function and utilization of iron in the body. Furthermore, oxidative stress, inflammation, bone marrow depression, and gastritis caused by smoking have been shown to result in anemia indicative of low Hb and Hct [40].

Hemoglobin is a protein in the red blood cells, while hematocrit reflects the percentage of blood volume that is composed of red blood cells. It is well known that Hb and Hct correlate with one another and both

are excellent correlates of anemia [41]. The greater Hct value among smokers was therefore expected since our result also indicated lower Hb concentration among the smokers compared with non-smokers. Interestingly, our study indicated significant correlation between Hb and Hct. In view of the above, it is expected also that the interplay of same factors associated with smoking, which affected Hb concentration, such as low serum ferritin levels, decrease in levels of vitamin C, Vitamin B12 and folic acid, as well as smoke-induced oxidative stress, inflammation, bone marrow depression, may also explain the lower Hct level found in smokers in the present study.

Among the smokers, Hb and Hct did not increase with increase in duration of smoking habit. A previous study by Acik et al indicated that there was no significant correlation between duration of cigarette smoking and Hb levels [30]. Another study by Inal et al [42] also reported that when smokers classified based on smoking duration of <2 and =2 years, Hb, Hct, values did not differ between the groups. These studies therefore are in agreement with the present finding. We did not find any significant differences in Hb and Hct on the basis of intensity of smoking. However, other studies have observed that haemoglobin concentration and hematocrit were significantly increased with increase in number of cigarettes smoked per day [17, 24, 30, 43]. Our study indicated no significant differences in Hb and Hct based on age of smokers - older smokers (25 years) vs. younger smokers (<25 years). Some previous studies have reported that haemoglobin concentration increases with increasing age in cigarette smoking men [33, 44]. Another study by Tirlapur et al [45] further demonstrated that in smokers, alcohol consumption >14 drinks/week and more than seven drinks/week for men and women, respectively, increased mean haemoglobin by 1.3% in men and by average 1.9% in women compared with those consuming =14 and less than or equal to seven drinks/week. In our study, no significant differences were observed in mean Hb and Hct between smokers who drink alcohol and those who do not. To the best of our knowledge, no previous study has been done on the effects of current smoking status (daily smoking vs. occasional smoking) and family history of smoking on Hb and Hct levels. In the present study, we observed no significant differences in Hb and Hct based on the subjects' current smoking statuses and family history of cigarette smoking respectively.

The limitations of this study included limited sample size used in the study, and our inability to obtain any information about what brands of cigarettes were consumed by the smoker group or about their preferred smoking methods. In addition, we could not quantify the effects of other cigarette smoke-induced factors such as serum levels of iron and ferritin, vitamin C, Vitamin B12 and folic acid levels, as well as smoke-induced oxidative stress, in order to understand their roles in the lower Hb and Hct observed among the smokers.

CONCLUSION

The present study indicated significantly higher mean COHb, but lower Hb and Hct levels among current smokers compared with non-smoking control. Furthermore, Hb and Hct levels were not affected by the duration of smoking, current smoking status of smokers, intensity of smoking, age of smokers, alcohol habits of smokers and family history of smoking.

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Author contributions:

MAE and ABIG conceptualized and designed the study. GEO, DU, FOO, OVA, ONA and FOA contributed to implementation of the project and

revision of the manuscript. All authors were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agreed to be accountable for all aspects of the work.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest:

None declared.

Ethical approval:

The study was approved by the Institutional Ethics Committee.

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