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Effect of Exposure to Petroleum Products on Methaemoglobin Levels of Fuel Station Attendants in Port Harcourt, Nigeria.

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Abstract

The aim of this study was to investigate the effect of the associated hazards of exposure to petroleum products on methaemoglobin levels of fuel station attendants in Port Harcourt metropolis. Fuel station attendants are exposed to the hazards of their occupation through inhalation of petroleum fumes, ingestion by chance and through skin penetration from broken skin surfaces. A total of forty-nine (49) subjects consisting of twenty-nine (29) occupationally exposed fuel station attendants and twenty (20) non-exposed apparently healthy aged-matched controls were enrolled. K₃EDTA was used to collect 4ml of venous blood from all subjects. Methaemoglobin estimation was based on Evelyn and Malloy manual method. Measurement was done spectrophotometrically at 630nm wavelength and the results were reported as percentage. Statistical analysis was carried out using Graph-Pad Prism 5 and a significant difference in methaemoglobin level ($p < 0.0001$) was observed between fuel station attendants (5.676 ± 3.044) and control subjects (2.085 ± 0.815). Comparison based on body mass index and gender were made in relation to methaemoglobin levels and it was observed that none had a statistical significance on methaemoglobin levels ($p > 0.05$). Increased duration of occupational exposure to petroleum products was associated with increase in methaemoglobin level. This study shows that exposure to petroleum products are associated with changes in methaemoglobin levels and can potentially be detrimental to the health of fuel station attendants. There is need for measures to be put in place to reduce the risk of occupational exposure to petroleum products. The methaemoglobin levels and health status of occupational exposed individuals should be monitored regularly.

Keywords: Effect, Exposure, Petroleum Products, Methaemoglobin, Fuel Station Attendants, Port Harcourt, Nigeria

Introduction

In contemporary society human engages in different commercial occupations in order to earn a meaningful livelihood and also contribute their quota to economic growth and development. Fuel station attendants are workers that perform services that include the sale of petroleum products like premium motor spirit (PMS), automated gas oil (AGO), dual purpose kerosene (DPK) and lubricants. These products except lubricants are stored in underground storage tanks (USTs) and dispensed through pumps and dispenser nozzles into automobiles and other receptacles. Cleaning of windscreen and checking of vehicle oil levels are equally undertaken by these attendants.

Fuel is any material that store potential energy in forms that can practically release such energy for work in the form of heat or power (Hugh, 1995). Fuel contain chemical component after fractional distillation such as petroleum gases, gasoline (benzene), petroleum ether, gas oil, bitumen diesel oil, ligroin, lubricating oil, and other residues (Ababio, 2001).

In the course of performing their occupational responsibilities, fuel station attendants are exposed to fuel product (benzene, gasoline oil and others harsh product in fuel) by inhalation, accidental ingestion and skin contact particularly for those who immerse their hands in fuel. These chemicals constitute a potential threat despite the satisfaction they derive in terms of profit and services rendered (Metzger Law Group, 2012).

Exposure to petroleum products can lead to the production of methaemoglobin (MetHb) which differs from haemoglobin upon reaction with blood (Rowat, 1998). One of the occupational effects of benzene in human body is the production of methaemoglobin. Haemoglobin functions mainly by transporting oxygen when the iron atom is in its ferrous state (Fe^{2+}). As haemoglobin is oxidized as a result of exposure to benzene in petroleum products, iron is converted to the ferric state (Fe^{3+}) or to MetHb. MetHb lacks the electron that is necessary for bonding with oxygen, and as a result, transport of oxygen is impeded (Callister, 2003). Red blood cells are normally and continuously being exposed to various oxidant stresses, therefore, MetHb levels of between 1 and 3% are usually observed (Lee and Tuyl, 2017).

Fuel station attendants are constantly exposed to petrol vapours that contain benzene and as such, normal body physiology is affected, with the highest impact on the respiratory system (Uzma *et al.*, 2008). Petroleum products can cause significant respiratory symptoms like chronic cough, breathlessness or shortness of breath and wheezing (Singhal *et al.*, 2007). An exposure assessment study on petrol station attendants carried out in Calabar, Nigeria, indicated that MetHb levels were increased than in non-exposed individuals (Udonwa *et al.*, 2009). There is paucity of data on the MetHb levels among petrol station attendants who are occupationally exposed to petroleum products in Port Harcourt, Nigeria. The aim of this research was to investigate the effect of occupational exposure to petroleum products on MetHb levels among fuel station attendants in Port Harcourt, Nigeria.

Materials and Method

This was a population-based-case-control study in which MetHb levels were estimated among individuals who were occupationally exposed to petroleum products and compared with non-exposed controls.

Study Area and Population

The study was carried out within Port Harcourt metropolis. For fuel station attendants, eleven (11) of them were enrolled from Oando petrol station in Mile III; seven (7) from Mobil petrol station in Mile II; eight (8) from Conoil petrol station, Leventis bus stop in Mile I and three (3) from Forte petrol station, Olu Obasanjo road. The non-exposed controls were recruited from Rumueme, Port Harcourt where fuel stations are not located. A total number of 49 subjects, which consist of twenty-nine (29) fuel station attendants and twenty (20) non-exposed controls within the ages of 18-50 years, were recruited for the study.

Consent

Verbal informed consent was obtained from individuals recruited for the study and ethical approval were obtained from the head of the respective fuel stations.

Sample Collection

Four milliliters (4mls) blood sample was collected from each participant using standard venepuncture technique as described in a previous report (Jury *et al.*, 2012) into vacutainer EDTA bottle. After collection, the blood sample was sufficiently mixed with the anticoagulant to ensure complete homogeneous mixture of blood and EDTA anticoagulant. The container was labeled with the patient's name, gender and age. The samples were transported to the laboratory for analysis within 1 hour of collection, using thermo-container without ice blocks for maintaining a temperature between $18^{\circ}\text{C} - 24^{\circ}\text{C}$.

Haematological Analysis

Haematological analysis for methaemoglobin measurement was carried out based on Evelyn and Malloy (1938) standard manual method.



Principles for Determination of Methaemoglobin

Methaemoglobin (Hi) has a maximum absorption at 630nm. When cyanide is added, this absorption band disappears and the resulting change in absorbance is directly proportional to the concentration of Hi. Total haemoglobin in the sample is then measured after complete conversion to HiCN by the addition of ferricyanide – cyanide, reagent. The conversion will measure oxyhaemoglobin and methaemoglobin but not sulphaemoglobin. Thus, the presence of a large amount of sulphaemoglobin will result in an erroneously low measurement of total haemoglobin. Non-ionic detergent was added to the haemolysate to avoid turbidity.

Reagents

Phosphate buffer: 0.1mol/L, pH 6.8

Potassium cyanide: 50g/L

Potassium ferricyanide: 50g/L

Non-Ionic Detergent: 10g/L

Laboratory Procedure

EDTA anticoagulated blood (0.2ml) was lysed in a solution containing 4ml of buffer and 6ml of detergent solution and the lysate was divided into 2 equal volumes (A and B). The absorbance of A was measured with a spectrophotometer at 630nm wavelength and recorded as (D_1). One drop of potassium cyanide solution was added and the absorbance measured again, after mixing and recorded as (D_2). One drop of potassium ferricyanide solution was added to tube B, and after 5 minutes, the absorbance was measured at the same wavelength and recorded as (D_3). Finally, one drop of potassium cyanide solution was added to tube B and after mixing a final reading (D_4) was obtained.

All the measurements were made against a blank containing buffer and detergent in the same proportion as present in the sample.

Calculation:

$$\text{Methaemoglobin Hi (\%)} = \frac{D_1 - D_2}{D_3 - D_4} \times 100$$

Data Analysis

Data were analyzed using GraphPad Prism 5 to obtain values for descriptive statistics (mean and standard deviation) and values for inferential statistics (t-test). The sample populations were grouped into fuel attendants and controls. Student t-test was used to test for difference between fuel attendants (subjects) and non-exposed controls. An error probability ($p \leq 0.05$) was considered statistically significant. Results of methaemoglobin level were reported in percentages.

Results

The demographic details of those recruited in the study are shown in Table 1, with records of calculations body mass index (BMI) gotten from their heights and weights. Making a comparison of mean methaemoglobin concentration between the fuel station attendants and non-exposed controls, it was observed that there was a statistically significant increase of methaemoglobin among fuel station attendants compared to non-exposed controls as shown in Table 2. Body mass index and gender have no effect on the methaemoglobin level as seen in Table 3 and Table 4 respectively. Based on the number of years the participants have worked as fuel station attendants, it was observed that there was increase in methaemoglobin levels as years increases as shown in Table 5.

Table 1: Demographic details of participants

S/N	Parameter	Number of Person/Age/Values of Parameters
1	Total number of subject	49
2	Total number of fuel station attendant	29(12 females,17males)
3	Total number of control subject	20 (10 males, 10 females)
4	Age range (Years)	18-55
5	Weight range (Kilograms)	50-98
6	Height range (Metres)	4.2-6.5
7	Range of body mass index (BMI)	10.0 – 30.0

Table 2: Comparison of mean ± SD of methaemoglobin level between fuel station attendants and control subjects

Parameter	Fuel station attendant (n =29)	Controls (n=20)	p- value
Methaemoglobin (%)	5.676 ± 3.044	2.085 ± 0.815	≤ 0.0001

Table 3: Comparison of mean ± SD of body mass index (BMI) of fuel attendant in relation to their methaemoglobin level

BMI	10.81 – 15.29 (n= 26)	16 & above (n =3)	P value
Methaemoglobin (%)	6.012 ± 2.892	2.767 ± 2.914	0.2091

Table 4: Comparison of mean ± SD of methaemoglobin level between female and male fuel station attendants

Parameter	Male (n=17)	Female (n =12)	P value
Methaemoglobin (%)	5.905 ± 3.154	5.35 ± 2.992	0.6348

Table 5: Comparison of mean ± SD of fuel attendant’s methaemoglobin levels based on number of year of exposure to fuel pollutant in relation to their methaemoglobin level

Parameter	(n= 17) 1 – 5yrs	(n =10) 6 – 10yrs	(n = 2) 11yrs & above	P value
Methaemoglobin (%)	3.59± 1.97	8.2 ± 0.79	10.8 ± 1.131	0.0001



Discussion

In this research, the level of methaemoglobin among fuel station attendants was compared with that of the controls. Result obtained indicates that there is a significant increase ($p < 0.0001$) in the methaemoglobin level in fuel station attendants than in the controls. The methaemoglobin level among fuel attendants obtained in this research agrees with the finding in a previous report (Denshaw-Burke *et al.*, 2016) which indicated methaemoglobin levels of 1–2%. Exposure to toxic chemical such as exhaust fumes, dapson, benzene, nitrobenzene, aniline, benzocaine, etc, increases methaemoglobin level significantly. The findings of this study also agree with that of Udonwa *et al.*, (2009) in Calabar, Nigeria; as well as that of Christian *et al.*, (2015) where exposure to sources that increase methaemoglobin levels in Abattoir workers had adverse effect on them when compared to controls.

Individuals are occupationally exposed to benzene and others toxic chemicals either by inhalation, ingestion or through skin contact with petrol products (Rowat, 1998). In this study, we observed that the level of methaemoglobin among fuel attendant increases with the number of years fuel station attendants. Our finding is consistent with previous reports (Christian *et al.*, 2015; Udonwa *et al.*, 2009).

Body mass index and gender of fuel station attendants did not have any statistically significant effect on the levels of methaemoglobin as a result of their exposure to benzene. This finding also agrees with that of Christian *et al.* (2015) with regards to number of years of exposure.

Conclusion

This study has shown that exposure to petroleum products can increase methaemoglobin levels in humans. Increased methaemoglobin can be used as a diagnostic marker of the toxic effect of benzene present in petroleum product relative to occupational

exposure. It is therefore necessary to take into cognizance that components in petroleum products, especially benzene, are capable of causing changes in methaemoglobin levels that are detrimental to the health of fuel station attendants and hence the need for measures aimed at preventing these worker from getting exposed to petroleum fumes by wearing protective equipment (nose mask; preventing skin contacts by wearing of thick rubber hand gloves, covered rubber shoes, and protective clothing).

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