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Effects of gasoline on haematological parameters of gasoline station workers in Onitsha, Anambra State, Nigeria.

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Abstract

Gasoline station workers (GSWs) are occupationally exposed to gasoline every day. Our previous study on the toxicological and biochemical profile of GSWs in Onitsha, Anambra state revealed that GSWs are at risk of benzene and lead-mediated toxicity. This was because the GSWs had increased methaemoglobin (used as a biomarker to measure benzene) and blood lead levels. The aim of this paper was to assess the effects of gasoline on the haematological parameters of GSWs in Onitsha, Anambra State, Nigeria. The blood samples of 160 subjects (80 GSWs and 80 controls) were tested for the full blood count (FBC) which includes the total white blood cell count, red blood cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count, haemoglobin concentration and packed cells volume. Questionnaires were distributed to the GSWs in order to ascertain their duration of exposure to gasoline. The GSWs were divided into three: group A (those exposed for 1 to 6 months) (n=34); group B (those exposed for 6 months to 2 years) (n=22); group C (those exposed for over 2 years) (n=24). Two milliliters of whole blood were collected by venepuncture into EDTA anticoagulated tube and used for the FBC. The FBC was performed within 24 hours of sample collection. Ethical approval was obtained from the ethics committee of the Nnamdi Azikiwe University, Nnewi, Anambra state while the informed consent of the participants was duly sought and obtained. The FBC was analyzed by flow cytometry using the Sysmex Autoanalyzer.

The RBC count, MCH, MCHC, PCV and platelet count were significantly reduced in the GSWs when compared with the controls ($p < 0.05$). The RBC count, WBC count, haemoglobin concentration, MCH, MCHC and PCV were highest among group A participants and lowest among group C participants ($p < 0.05$). RBC count, MCH, MCHC, PCV and platelet count were significantly reduced in the test subjects compared to the controls and this could imply ongoing anaemia among the GSWs. The Red Blood Cell and White Blood Cell counts, Haemoglobin, MCH, MCHC and Packed Cells Volume were highest among group A workers and lowest among group C. These findings, just like in the previous one, also suggests that GSWs are highly exposed to toxic gasoline vapor, and reduced haematological parameters among the workers exposed for longer durations suggesting the presence of iron deficiency anaemia and leukocytopenia among these subjects which could consequently affect red blood cell production, functions as well as immunity.

Keywords: *Gasoline Station workers, occupationally exposed, Benzene and Lead Toxicity, Haematological parameters.*

Introduction

Gasoline popularly called Petrol in this part of the world is a complex mixture of hydrocarbons, produced by mixing fractions obtained from the distillation of crude oil with brand specific additives to improve performance (Ezejindu *et al.*, 2014). Under normal conditions, it is a volatile liquid with a characteristic odour.

Premium Motor Spirit (PMS) is mainly used as a fuel for light road vehicles (cars, motorbikes, tricycles and small vans) and small appliances (lawnmowers, cement mixers etc).

Gasoline station workers, who are employed to dispense gasoline in filling stations are frequently exposed to different petroleum derivatives which include the aromatic hydrocarbon benzene and the metal lead, both of which have deleterious effects on human health (Okoro *et al.*, 2006). Benzene is widely recognized as a human carcinogen which can induce certain diseases of the blood like leukaemia, anaemia and coagulopathy (Smith, 1996). It is present in the environment in water, soil and air. Some of the major sources of benzene are gasoline, car exhaust, paint, and glues. Approximately 95% of exposure to benzene is through inhalation (Duarte-Davidson *et al.*, 2001).

Lead is one of the most toxic substances that large populations around the world are exposed to from the exhaust gases of vehicles and other sources. Leaded gasoline is known to have caused more exposure to lead than any other known source (Landrigan, 2002). Although many industrialized countries have banned the use of lead in gasoline its use is still prevalent among less developed nations (Al-Rudainy, 2010). Lead in gasoline stations where GSWs work comes from emissions in the form of fine particles that are inhaled and absorbed through the lungs, by ingestion, and through dermal exposure (Herman *et al.*, 2006). Within the body, it is distributed to the brain, liver, kidney and bones (Akumu, 2012). Lead and other components of gasoline have been shown to exhibit harmful effects on the bone marrow, spleen and lymph nodes (Marieb, 1995).

Several authors have documented the adverse effects of benzene and Lead (used as gasoline additives) on some haematological parameters. Our previous study (Obi *et al.*, 2017) revealed the presence of elevated blood Lead and methaemoglobin (benzene biomarker) levels among gasoline station workers who had prolonged occupational contact with gasoline. Sahb (2011), using the complete blood count to

assess benzene toxicity, reported reduced Haemoglobin (Hb), White Blood Cell (WBC) and Red Blood Cell (RBC) counts among GSWs compared to their controls. Ajugwo *et al.* (2014) also reported reduced PCV, Hb, Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) among GSWs, an indication pointing towards the onset of anaemia. Tunsaringkarn *et al.* (2013) reported reduced Hb, PCV and eosinophil counts among GSWs. Santiago *et al.* (2014) reported low platelet counts in GSWs. Al-Rudainy (2010) reported blood lead levels higher than the upper lead limit for adults. This study was embarked upon to measure haematological parameters among GSWs. This is because reductions in the use of lead in petrol have resulted in substantial reductions in Lead levels in blood (Fewtrell *et al.*, 2003). The findings of the study were intended to reveal the adverse effects of continuous contact with petrol by GSWs as published in previous studies. No prior study on the subject had been carried out in the state, therefore this study was carried out to assess the effects of gasoline on the haematological parameters of gasoline station workers in Onitsha, Anambra State.

Materials and Methods

Subjects

The eighty (80) subjects in this study were selected from different petrol stations across Onitsha metropolis while the controls who were also eighty (80) in number were made up of students and healthy individuals residing in the same metropolitan area. Study subjects were divided into three groups: group A comprised those exposed for one to six months (n=34); group B were made up of those who had been exposed for six months to two years (n=22); while group C consisted of those with over two years exposure (n=24).

Sample Collection

Two millilitres (2 mls) of blood was collected from each subject by clean venepuncture into sample tubes containing di-potassium salt of ethylenediamine tetra-acetic acid (K₂ EDTA) at a concentration of 1.2mg/ml of blood. Full blood count (FBC) was determined using the SYSMEX KX-21 Haematology autoanalyzer. FBC

parameters comprising the red blood cell count, haemoglobin concentration, red cell indices, total white blood cell count, packed cells volume and platelet count were determined. Ethical approval and written informed consent of the subjects were duly sought and obtained prior to commencement of the study. The study was carried out at the facilities of Iyieniu Mission Hospital Ogidi, located in Anambra state, Nigeria.

Statistical Analysis

This study was designed in a completely randomized method and data collected were analyzed by the one-way analysis of variance (ANOVA) in order to test for the significance of the difference among groups, while the students' t-test was used to test for the significance of the difference between groups using the statistical package for social sciences (SPSS) software application (version 20). The multiple comparisons were made using the Post- hoc test. The accepted level of significance was set at $p < 0.05$.

Results

Table 1 shows significant decrease in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) ($p < 0.05$) among gasoline station workers compared to control. In addition, there was a significant decrease in the red blood cell count (RBC), packed cells volume (PCV) and platelet count among the subjects when compared with the controls. The pair-wise comparison (Table 2) was made among those who had been exposed for one to six months (group A), those who had been exposed for six months to two years (group B) and those with over two years exposure (group C). The post-hoc test showed a significant decrease in the RBC, white blood cell count (WBC), haemoglobin concentration and PCV with respect to duration of exposure ($p > 0.05$ in each case).

Table 1: Haematological parameters between the gasoline station workers and the control subjects

Parameters	Test (n=80)	Control (n=80)	p-value
RBC ($\times 10^{12}/l$)	4.47±0.72	<0.001*
Hb (g/dl)	11.73±1.46	11.99±1.56	0.300
MCV (fl)	81.63±8.79	83.27±6.78	0.138
MCH (pg)	28.90±5.39	36.20±5.66	<0.001*
MCHC (g/dl)	29.32±4.23	39.96±4.56	<0.001*
WBC ($\times 10^9/l$)	5.46±1.46	5.82±1.57	0.128
PCV (%)	29.25±3.85	37.49±4.96	<0.001*
Platelet ($\times 10^9/l$)	191.55±37.83	237.60±77.52	<0.001*

*p-value is significant at 0.05.

Key: RBC=red blood cell count; Hb=haemoglobin concentration; MCV=mean cell volume; MCH=mean cell haemoglobin; MCHC=mean cell haemoglobin concentration; WBC=total white blood cell count; PCV=packed cells volume; Platelet=platelet count.
 Test=gasoline station workers; Control=control subjects.

Table 2: Haematological parameters among gasoline station workers based on duration of exposure using one-way ANOVA and post-hoc

Parameters	Group A (n=34)	Group B (n=22)	Group C (n=24)	F-value	p-value
RBC (X10 ¹² /l)	3.802±0.298	3.546±0.336	3.358±0.488	10.232	<0.001*
Hb (g/dl)	12.147±1.338	11.718±1.628	11.150±1.320	3.478	0.036*
MCV (fl)	81.447±7.474	80.114±10.487	83.279±8.917	0.753	0.474
MCH (pg)	32.388±3.599	30.391±4.364	22.604±1.532	0.324	0.725
MCHC (g/dl)	32.168±2.730	30.682±2.209	24.033±1.846	0.386	0.681
WBC (x10 ⁹ /l)	5.753±1.421	5.673±1.453	4.842±1.405	3.222	0.045*
PCV (%)	30.935±3.406	28.336±4.196	27.713±3.272	6.618	0.002*
Platelet (x10 ⁹ /l)	190.029±36.061	189.909±43.170	195.208±36.462	0.157	0.855
POST-HOC	A/B	A/C	B/C		
RBC (x10 ¹² /l)	0.014*	<0.001*	0.093		
Hb (g/dl)	0.272	0.010*	0.179		
MCV (fl)	0.582	0.438	0.227		
MCH (pg)	0.034*	<0.001*	<0.001*		
MCHC (g/dl)	0.024*	<0.001*	<0.001*		
WBC (x10 ⁹ /l)	0.838	0.019*	0.052		
PCV (%)	0.010*	0.001*	0.559		
Platelet (x10 ⁹ /l)	0.991	0.613	0.640		

*p-value is significant at 0.05. N=80.

Key: RBC=red blood cell count; Hb=haemoglobin concentration; MCV=mean cell volume; MCH=mean cell haemoglobin; MCHC=mean cell haemoglobin concentration; WBC=total white blood cell count; PCV=packed cells volume; Platelet=platelet count.

Group A= gasoline station workers exposed for a duration of 1 to 6 months; Group B= gasoline station workers exposed for a duration of 6 months to 2 years; Group C= gasoline station workers exposed for a duration of over 2 years

Discussion

The present study which is a follow-up from our previous study (Obi *et al.*, 2017), was undertaken to assess the effects of gasoline on the haematological parameters of gasoline station workers in Onitsha, Anambra state, Nigeria.

In our previous study (Obi *et al.*, 2017), we reported an increase in the methaemoglobin level of the gasoline station workers, reflecting a high risk of benzene exposure and elevated blood lead levels in the subjects. Observation from this present study shows that the red blood cell (RBC) count was significantly reduced in the gasoline station workers compared with their

controls. This possibly stems from the fact that when lead enters the vasculature, it will induce changes in the composition of RBC membrane proteins and lipids (Balleova *et al.*, 2001). This could predispose the RBCs to haemolysis due to their altered membrane. The biconcave shape of the RBC enables its deformability and ability to squeeze through capillaries, and also aids in the reduction of tension in the cell wall as the cell swells after taking up CO₂ from the tissues (Bailey and Gwinnutt, 2008). Once the RBC membrane is altered, it loses these properties and, as earlier noted, becomes susceptible to haemolysis and consequently, anaemia.

The mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were significantly reduced in the PPAs than in their controls. This could be as a result of the body trying to compensate for a rapidly progressing anaemic state. There was no significant difference in the Haemoglobin concentration of the PPAs when compared with the controls. This could be due to the fact that even though some of the subjects were anaemic, they might have physiologically developed some sort of compensatory mechanism (as suggested above) to sustain oxygen unloading to tissues from Haemoglobin despite its reduced concentration in the body (Erslev, 1983; Pollock and Cotter, 1973). This finding however does not agree with the findings of Ajugwo *et al* (2014) and Sahb (2011), but suggests that the haemoglobin concentration may not be a reliable index to be solely used for assessing anaemia in PPAs. There was also no significant difference between the total white blood cell (WBC) count of the study subjects compared to the controls probably because majority of the test subjects had been exposed to petroleum products for less than six months (42.5%). However, the platelet count in the PPAs was significantly reduced in the test subjects in comparison to the controls. This agrees with previous reports (Santiago *et al.*, 2014; Qu *et al.*, 2002; Aksoy *et al.*, 1987) and could probably be as a result of the suppressive effects of benzene on the bone marrow and consequently, thrombopoiesis.

Subjects exposed to gasoline fumes beyond two years had lower RBC, haemoglobin level, WBC count and PCV respectively than those exposed for two years or less as shown in Table 2. Comparisons based on duration of exposure showed that the RBC count and PCV of test subjects exposed for one to six months were significantly lower than the values of those exposed for up to two years or more. It was also observed that the haemoglobin concentration and WBC count of those who had been exposed for one to six months were significantly lower when compared to the values of those who had been exposed for more than two years. These findings agree with the findings in previous reports (Ismail *et al.*, 2006; Okoro *et al.*, 2006; Ajugwo *et al.*, 2014).

Conclusion

The findings of this study suggest that haematological parameters in petrol station attendants are adversely affected by gasoline exposure, with a decline in the red blood cell count, packed cells volume and platelet count suggesting the presence of anaemia and thrombocytopenia in gasoline station workers. A reduced WBC count observed in workers chronically exposed to gasoline fumes suggests the possibility of impaired immunity in such persons.

Conflict of Interest Declaration: The authors hereby declare that they have no conflict of interest.

Authors' Contributions: AFA, EO and MOI designed the protocol. AFA acquired and analyzed data. AFA, EO, MOI and CIE executed laboratory and bench work. All were involved in preparation of the manuscript.

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