Effects of Gasoline on Haematological Parameters of Gasoline Station Workers in Onitsha, Anambra State, Nigeria.

By

Arinze F. Anyiam *, Ejeatuluchukwu Obi **, Martin O. Ifeanyichukwu *** and Cajetan Ilo Elochukwu **

ABSTRACT
Gasoline station workers (GSWs) are occupationally exposed to gasoline daily. 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). Blood sample of 2 mls volume was collected by venepuncture 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 analysed 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 vapour, and reduced haematological parameters among the workers exposed for longer durations suggest the presence of iron deficiency anaemia and leukocytopenia among these subjects which could consequently affect red blood cell production and functions, and also immunity.

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

* Corresponding author: Email: arinzeanyiam@gmail.com ; Tel: +2347035349878
* Author’s address: Department of Medical Laboratory Science, Haematology Unit, Faculty of Pure and Applied Sciences Kwara State University, Malete, P. M. B. 1530, Kwara State, Nigeria.
** Co-Author’s address: Toxicology Unit, Department of Pharmacology and Therapeutics, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus,Nnewi, Anambra State,Nigeria.
*** Co-Author’s address: Department of Medical Laboratory Science, Immunology Unit, Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus, P. M. B. 5001, Anambra State, Nigeria.
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. Under normal conditions, it is a volatile liquid with a characteristic odour.

Gasoline 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. Benzene is widely recognized as a human carcinogen which can induce certain diseases of the blood like leukemia, anaemia and coagulopathy. 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.

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. Although many industrialized countries have banned the use of lead in gasoline its use is still prevalent among less developed nations. 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. Within the body, it is distributed to the brain, liver, kidney and bones. Lead and other components of gasoline have been shown to exhibit harmful effects on the bone marrow, spleen and lymph nodes.

Several authors have documented the adverse effects of benzene and lead (used as gasoline additives) on some haematological parameters. Our previous study revealed the presence of elevated blood Lead and methaemoglobin (benzene biomarker) levels among gasoline station workers.

---

workers who had prolonged occupational contact with gasoline. Sahb 11 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 12 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 13 reported reduced Hb, PCV and eosinophil counts among GSWs. Santiago et al 14 reported low platelet counts in GSWs. Al-Rudainy 15 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 gasoline have resulted in substantial reductions in Lead levels in blood16. The findings of the study were intended to reveal the adverse effects of continuous contact with gasoline 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 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 and was immediately transported in ice packs to the analytical laboratory.

The samples were dispensed into bottles containing the di-potassium salt of ethylenediamine tetra-acetic acid (K2 EDTA) at a concentration of 1.2mg/ml of blood and the SYSMEX KX-21 haematology autoanalyzer was used to determine the full blood count (FBC) comprising the red blood cell count, haemoglobin concentration, red cell indices, total white blood cell count, packed cells volume and platelet count. Ethical approval and informed consent of the subjects were duly

15 op. cit. See note 6 above
sought and obtained prior to commencement of the study. The experiments were carried out using the facilities of Iyienu Mission Hospital Ogidi, located in Anambra state, Nigeria.

**STATISTICAL ANALYSIS**

The experiments were 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 red blood cell count (RBC), packed cells volume (PCV), platelet count, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) (p<0.05) compared between gasoline station workers and control subjects. 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, MCH, MCHC and PCV with respect to duration of exposure (p>0.05 in each case).

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test (n=80)</th>
<th>Control (n=80)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X10^{12}/l)</td>
<td>3.60±0.42</td>
<td>4.47±0.72</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.73±1.46</td>
<td>11.99±1.56</td>
<td>0.300</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>81.63±8.79</td>
<td>83.27±6.78</td>
<td>0.138</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.90±5.39</td>
<td>36.20±5.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>29.32±4.23</td>
<td>39.96±4.56</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>WBC (X10^{9}/l)</td>
<td>5.46±1.46</td>
<td>5.82±1.57</td>
<td>0.128</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>29.25±3.85</td>
<td>37.49±4.96</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Platelet (X10^{9}/l)</td>
<td>191.55±37.83</td>
<td>237.60±77.52</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* 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.
### Effects of Gasoline on Haematological Parameters

#### TABLE 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (n=34)</th>
<th>Group B (n=22)</th>
<th>Group C (n=24)</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X10^{12}/l)</td>
<td>3.802±0.298</td>
<td>3.546±0.336</td>
<td>3.358±0.488</td>
<td>10.232</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.147±1.338</td>
<td>11.718±1.628</td>
<td>11.150±1.320</td>
<td>3.478</td>
<td>0.036*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>81.447±7.474</td>
<td>80.114±10.487</td>
<td>83.279±8.917</td>
<td>0.753</td>
<td>0.474</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>32.388±3.599</td>
<td>30.391±4.364</td>
<td>22.604±1.532</td>
<td>0.324</td>
<td>0.725</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32.168±2.730</td>
<td>30.682±2.209</td>
<td>24.033±1.846</td>
<td>0.386</td>
<td>0.681</td>
</tr>
<tr>
<td>WBC (X10^{9}/l)</td>
<td>5.753±1.421</td>
<td>5.673±1.453</td>
<td>4.842±1.405</td>
<td>3.222</td>
<td>0.045*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>30.935±3.406</td>
<td>28.336±4.196</td>
<td>27.713±3.272</td>
<td>6.618</td>
<td>0.002*</td>
</tr>
<tr>
<td>Platelet (X10^{9}/l)</td>
<td>190.029±36.061</td>
<td>189.909±43.170</td>
<td>195.208±36.462</td>
<td>0.157</td>
<td>0.855</td>
</tr>
</tbody>
</table>

**POST-HOC**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A/B</th>
<th>A/C</th>
<th>B/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X10^{12}/l)</td>
<td>0.014*</td>
<td>&lt;0.001*</td>
<td>0.093</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>0.272</td>
<td>0.010*</td>
<td>0.179</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>0.582</td>
<td>0.438</td>
<td>0.227</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>0.034*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>0.024*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>WBC (X10^{9}/l)</td>
<td>0.838</td>
<td>0.019*</td>
<td>0.052</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>0.010*</td>
<td>0.001*</td>
<td>0.559</td>
</tr>
<tr>
<td>Platelet (X10^{9}/l)</td>
<td>0.991</td>
<td>0.613</td>
<td>0.640</td>
</tr>
</tbody>
</table>

* 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\textsuperscript{17}, was undertaken to assess the effects of gasoline on the haematological parameters of gasoline station workers in Onitsha, Anambra state, Nigeria.

\textsuperscript{17} Op. Cit. See note 10 above.
In our previous study\(^\text{18}\), 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 same subjects.

From this present study, 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\(^\text{19}\). 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 CO\(_2\) from the tissues\(^\text{20}\). Once the RBC membrane is altered, it loses these properties and, as earlier noted, becomes susceptible to haemolysis and consequently, anaemia. The packed cells volume (PCV), Haemoglobin concentration, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were significantly reduced in the gasoline station workers than in their controls. These could be due to the effects of benzene as reported by D’Azevedo et al\(^\text{21}\).

Benzene causes pancytopenia and could lead to bone marrow aplasia. These findings agree with those of Dede and Kagbo\(^\text{22}\), and Ovuru and Ekweozor\(^\text{23}\). The toxic components of petroleum fumes have been reported to change blood chemistry and induce anaemia by causing bone marrow hypoplasia in experimental animals.\(^\text{24}\) This study suggests a similar effect on humans. Benzene and lead are toxic constituents of petrol. They become activated in the bone marrow and the cytotoxic effects observed are mediated through disturbance in DNA function\(^\text{25}\). The resultant bone marrow depression is characterized by inadequate production of red cells and other formed elements\(^\text{26}\). This correlates with the findings in this study.

The platelet count was also significantly reduced in the test subjects in comparison to their controls. This agrees with the findings of Santiago, et al\(^\text{27}\), Ismail et al\(^\text{28}\) and Qu et al\(^\text{29}\), and could probably

---

\(^\text{18}\) Ibid
\(^\text{25}\) Ajugwo et al. Op. Cit. See note 12 above
\(^\text{27}\) Op. cit. See note 14 above
Effects of Gasoline on Haematological Parameters

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, MCH, MCHC, WBC count and PCV respectively than those exposed for two years or less (Table 2). Comparisons based on duration of exposure showed that the RBC count, Haemoglobin concentration, MCH, MCHC and PCV of test subjects exposed for two years or more were significantly lower than the values of those exposed for one to six months. This could be due to the development of iron deficiency anaemia. It was also observed that the WBC count of those who had been exposed for two years or more were significantly lower when compared to the values of those who had been exposed for one to six months. This could imply a decline in the immunity of GSWs as period of exposure to gasoline increases. These findings agree with those of Okoro, et al\textsuperscript{30}, Aksoy et al,\textsuperscript{31} and Ajugwo, et al\textsuperscript{32}.

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, Haemoglobin concentration, MCH, MCHC 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.

COMPETING INTERESTS: The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS: AFA, EO and MOI designed the protocol. AFA acquired and analysed data. AFA, EO, MOI and CIE executed laboratory and bench work. All were involved in preparation of the manuscript.

ACKNOWLEDGMENTS: The authors acknowledge Obi Collins for the technical assistance provided for this study.

\textsuperscript{30} Op. cit. See note 2 above
\textsuperscript{32} Op. cit. See Note 12 above