**Blood Lead Level and its Effects on Occupationally Exposed Workers in Abeokuta, Nigeria**

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**ABSTRACT**

A study on the hazards of lead on environmentally exposed 80 male petrol station attendants and 35 male University students (who are residents of Abeokuta, an urban city in Nigeria) has been investigated by spectrophotometric analysis of their blood with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm, pH 7.4 and at 37°C. DTNB permits the determination of sulphydryl groups in simple compounds and has been used to monitor the disappearance of free SH groups in hemoglobin (Hb). The concentration of lead in the blood of each sample was also determined. The concentration values ranged from for the petrol station attendants 21.40 to 70.51 µg/dl and 14.23 to 17.65 µg/dl for the University students who served as the control. The mean values of blood lead concentration were found to be 41.36±2.71µg/dl for petrol station attendants, while that of the control is 14.31±2.16µg/dl.

The mean blood lead values of the petrol station attendants were significantly higher than that of the control (P< 0.05). At 37°C, the lead-hemoglobin reacted with DTNB resulting in decrease in the number of sulphydryl group (ß93-SH) per hemoglobin molecule. The values range from 3.8 to 5.6 -SH groups for the petrol station attendants and 4.6 – 5.8 -SH groups per tetramer for the control. The mean values are 4.8±1.67 –SH and 5.5±2.40 groups per Hb molecule for the petrol station attendants and the University students (control) respectively. This study demonstrates a predominant elevated blood lead levels in petrol station attendants in Abeokuta City, Nigeria and that atmospheric lead represents a significant source of human exposure to environmental pollutants in any city. These results also show differences in the reactivity of sulphydryl groups in the hemoglobin of this subject population. It is suggested that these differences arise from the variations in the level of exposure to lead, the environment of their ß93 sites, age, drinking and feeding habit, smoking status, as well as the level of education of the subject.

**Key words:** Hemoglobin - lead - sulphydryl group - petrol attendants - blood.

**INTRODUCTION**

Lead has been regarded as a toxicant for many years and remains a persistent environmental health threat. It enters into human body system through many ways and its effects are enormous (sdell *et al.*, 1972; Flora, 2000). Most human exposure to lead occurs via injection but in some cases, inhalation is the mode of entry into the body. Almost all inhaled lead is absorbed. Once in the body, the kidneys and liver rapidly excreted lead. Absorbed lead that is not excreted gets distributed into blood, soft tissues and bones.

Approximately, 99% of the lead in blood is associated with red blood cells. The remaining 1% residues is in the blood plasma (Ali, 1997; Carpenter D.O, 2001; Charkson T.W., 1987; Zou *et al.*, 1995).

Reports have shown that whenever lead enters the body system, it binds to cysteine residue of hemoglobin where it enhances or inhibit some certain enzymatic activities in the body. (Sdell *et al.*, 1972; Ye Xi-Biao *et al.*, 2003). Blood lead concentration has remained the most commonly used biomarker for ascertaining recent exposure.

ß-Amino levulinic acid dehydratase (ALAD) has been implicated as the principal binding site for lead in erythrocytes and it is encoded by a gene localized in human chromosome 9q 34 (Petrucci *et al.*, 1982; Sdell *et al.*, 1972). Earlier studies have shown that lead affects the production of hemoglobin and other heme-containing enzymes by displacing Zinc-dependent enzyme ALAD, suggesting that lead is a potent inhibitor of heme synthesis and a reduction in heme-containing enzyme could compromise energy metabolism and give rise to anemia (Clarkson, 1987; Zou *et al.*, 1995).

Hemoglobin is an oxygen-carrying molecule of the red blood cells of vertebrates. Leads bind to its cysteine residue through sulphydryl reaction (Sdell *et al.*, 1992).

In adult human hemoglobin, the free SH groups are located at position 93 of the ß-chains (Riggs A., 1965; Goldstein *et al.*, 1961) and are adjacent to the histidine at position 92, which are in close contact with the iron atoms of the hemes. Blocking these two SH groups affects the oxygen equilibrium of hemoglobin. (taylor *et al.*, 1966).

The need to remove the toxicant lead from the body system has been of interest to researchers in recent times. Ademuyiwa *et al.* (1994), reported the productive effects of Vitamin C in acetaminophen-induced hepatotoxicity. Onunkwor *et al.*, (2004), in their study, also concluded that Vitamin C was found to protect from the effects of lead in the plasma but fails in the tissue.

Lead has been classified as group 2B carcinogen in animals but however, sufficient data to support its role in human carcinogenesis are insufficient (Ali N.K., 1997). Although many studies have been carried out on the concentrations of lead in blood, kidney, liver, urine

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and soil (Astrin et al., 1987; Bergdahl et al., 1997; Ye Xi-Biao, et al., 2003; Lopes P., 2003; Ademuyiwa et al., 2002), it seems however that little is known on its effects on the sulfhydryl group reactivity of human hemoglobin. Additional studies are needed to determine the role of lead in sulfhydryl reactivity of the hemoglobin of exposed workers.

Therefore in the present paper, the environmental hazard of lead on occupationally exposed petrol attendants in Abeokuta, Ogun State, Nigeria has been examined in eighty workers, using their lead blood concentration as biomarker to correlate with the number of sulfhydryl groups, duration of exposure, blood pressure, and other social habits such as drinking and smoking.

**MATERIALS AND METHODS**

### Study Population

Participation in this study was voluntary and all the participants were informed of the research objective, study procedure and the potential risk. The study involves eighty (80) petrol station attendants and thirty five (35) students of the University of Agriculture, Abeokuta Nigeria (serving as control). The mean age of the attendants and the students were 32.37 ± 3.40 and 25.57± 2.61 years respectively. Blood pressure was measured two times in each subject at five minutes interval. A careful history of their dietary habit, age and their job experience was taken (Tables 1-5).

### Laboratory Analysis

The venous blood samples (10 mls each) were collected from eighty occupationally exposed petrol station attendants and also from thirty-five students (serving as control) for analysis. The hemoglobin was prepared according to standard laboratory procedure (serving as control) for analysis. The hemoglobin was prepared for the treatment. The DTNB used was a product of sigma st Louis, USA. Hemoglobin concentration of 4 µm tetramer in phosphate buffer 7.4, I = 0.05M was used throughout the experiment. The Hemoglobin concentrations were determined by Drabkins method as modified by Van Kampen and Zijlstra (1982).

The venous blood samples (10 mls each) were collected from eighty occupationally exposed petrol station attendants and also from thirty-five students (serving as control) for analysis. The hemoglobin was prepared according to standard laboratory procedure (Antonini and Brunori, 1971). The spectrophotomeric measurements were measured with α-heš́los pye unicam spectrophotometer and 2.57 mM DTNB stock solution was prepared for the treatment. The DTNB used was a product of sigma st Louis, USA. The hemoglobin samples were deionized by passage through sephadex G25 medium. The DTNB used was a product of sigma st Louis, USA. Hemoglobin concentration of 4 µm tetramer in phosphate buffer 7.4, I = 0.05M was used throughout the experiment. The Hemoglobin concentrations were determined by Drabkins method as modified by Van Kampen and Zijlstra (1982).

The effect of lead on sulfhydryl reactivity of the hemoglobin of the two categories was investigated, spectrophotomerically with DTNB. This compound permits the determination of sulfhydryl group in simple compounds (Okonjo and Okia, 1993; Ogunmola et al., 2002) and has been used to monitor the disappearance of free SH groups in hemoglobin following exposure to toxicants.

Varying amounts of DTNB (0.01 ml to 0.10 ml) were pipetted into the working tubes. 3 mls of distilled water were added into each tube and the mixture left to equilibrate for one hour after which the absorbance of the solution in each tube was read at 412nm (Absorption maxima for DTNB). This procedure was repeated for DTNB with aliquots 3mls of hemoglobin samples of the petrol attendants. Each experimental run was repeated twice at room temperature.

The sulfhydryl concentrations were determined by dividing the absorption maxima (Δmax) by the molar extinction coefficient 13,600 m⁻¹ cm⁻¹ assumed for 5 thio, 2-nitrobenzoate (TNB), the product of the reaction. The ratios of hemoglobin SH concentration to hemoglobin were calculated by dividing the appropriate values. The blood samples of the subjects were analyzed for the presence of lead on the Atomic Absorption Spectrophotometer (ALPHA AAS, Chemtech Analytical) at 217 nm. The blood samples were digested in Acid. Standard solutions of the lead were aspirated to calibrate the AAS before the inspiration of the samples.

### RESULTS

A study on environmental hazard of lead on occupationally exposed petrol station attendants has been investigated. Tables (1, 2 and 3) show job experience, ages and blood pressure of the subjects. The ages range between 18.5 to 30.1 and 19.7 to 38.4 for the University students and the petrol attendants respectively. The Mean ages of the petrol attendants are 32.37±3.40 while that of the University students had mean ages of 25.57±2.61 years. There is wide variation in the years of experience of petrol station attendants. This work is on male petrol station attendants because the attendants are predominantly male and they seem to stay more on the job.

The hemoglobin of the 115 subjects reacted with DTNB resulting to decrease in the number of free sulfhydryl group per hemoglobin molecule (Figures 1-3) represent the titration curves of the lead-hemoglobin with DNTB at 412 nm. Tables 1-3 present the biodata of subjects, blood lead level and its effects on occupationally exposed workers in Abeokuta, Nigeria.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Study Population</th>
<th>Job Experience in Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>University Student</td>
<td>M: 35</td>
<td>F: -</td>
</tr>
<tr>
<td>Petrol Attendants</td>
<td>80</td>
<td>0.17-23.00</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>9.25 ± 2.41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>University Student</td>
<td>18.5 – 30.1</td>
<td>25.57 ± 2.61</td>
</tr>
<tr>
<td>Petrol Attendants</td>
<td>19.7 – 38.4</td>
<td>32.37 ± 3.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Systolic</th>
<th>Diastolic</th>
</tr>
</thead>
</table>


Table (2): Age of the subjects in years.

Table (3): Blood pressure of the subjects (mmHg).
the petrol station attendants. In table 4, we report the results of the numbers of -SH groups in the samples. The number of -SH group ranges from 3.8 to 5.6 and 4.6 to 5.8 for the petrol station attendants and the students (control) respectively. The corresponding mean values are 4.8 ± 1.67 and 5.5 ± 2.30 for the petrol attendants and the students respectively.

The mean values of blood lead concentration were found to be 41.36 ± 2.71 for petrol station attendants, while that of the control is 14.31 ± 2.16 (Table 4). The mean blood lead values of the petrol attendants were significantly higher than that of the control (p < 0.05). The blood lead concentration of the petrol station attendants is about 3 times higher than that of the control.

The systolic blood pressures (Table 3) of the exposed petrol station attendants increases over that of the control. There is little difference in their diastolic blood pressure as presented in Table (3). This observation is in agreement with the studies of Goyer (1991) and Ademuyiwa et al. (2002) in their study on lead levels in the blood and urine of some residents of Abeokuta Nigeria.

**DISCUSSION**

Blood has been used extensively for the biological monitoring of the general population to lead exposure. Apart from urine, it is the biological most commonly used in monitoring exposure to heavy metals (Leighton et al., 2003; Ali N.K., 1997). Published studies had described blood lead levels less than 40 µg/dl as normal 40 – 80 µg/dl acceptable, 80-120 µg/dl excessive and greater than 120 µg/dl dangerous (Kazantzis G., 1988). The leaded hemoglobin of the petrol worker reacted with DTNB leading to a decrease in the number of -SH groups per hemoglobin molecule.

It is assumed in the present study that the extent of decrease in the number of sulphydryl groups is (indicator of the increase of) the level of the blood lead. Our results show differences in the reactivity of sulphydryl groups of hemoglobin of the 215 subjects examined. It is concluded that this variation, presumably arises from differences in tertiary structure around the β 93 sites of their beta-chain, and also in the duration of exposure to lead as well as their diets. Various reports have shown that lead binds to cysteine residue of hemoglobin through sulphydryl reaction due to the presence of lone pair of electron on sulphur and that the cysteine residue at position β 93 has the SH site for the reaction (Sdell et al., 1972).
It has also been demonstrated that lead irreversibly bonds with sulfhydryl group of proteins, causing impaired function without any discernible threshold (Demichele S.J., 1984). Delta-aminolevulinic acid dehydratase (ALAD) which is a catalyst for the formation of the porphobilinogen ring, and ferrochelatase, which is responsible for the insertion of iron into the protoporphyrin ring are both impaired by lead (Clarkson, T.W., 1987; DeMeichele, S.J., 1984; Sdell et al., 1972).

The results in Table (5) show that the hemoglobin from petrol station attendants has the lowest number of SH groups per hemoglobin molecule. This reduction in the number of SH groups of the petrol attendants may be due to the presence of lead as tetraethyl lead in the petrol (Silbergeld, 1996).

Vitamin C increases hemoglobin concentration and the number of free SH groups. Lead (Pb2+) binds to the lone pair of electron in the oxygen of vitamin C without attacking SH groups of the hemoglobin free (Wang, 1993). This vitamin has been found to reduce lead toxicity as it helps in the detoxification of lead (Ademuyiwa et al., 1994).

Our paper also indicates variation in the lowering of SH groups as a function of duration of exposure. The petrol attendants that have worked up to twenty years have lowest number of SH groups. This variation may be ascribed to the type of food or diet or medication that are being taken by these subjects. Lead gets into the body system through other means like drinking water, leaded pipe, smoking, combustion, paints, contaminated food, etc. Animal studies have shown that certain substances bind lead and increase its solubility thereby enhancing its absorption. As for example sodium citrate as ascorbate, amino acids, vitamin D, protein fat, and lactose increase lead solubility and hence its absorption (Demichele, 1984).

The higher number of sulfhydryl group in the blood of students (control) may be due to their feeding habit, the hygienic and conducive academic environment they stay. However the decrease in SH group of some of the subjects in the control to 4.6 may be as a consequence of the students being exposed to lead through other sources different from leaded petrol. Most human exposure to lead occurs via ingestion but in some cases, inhalation is the mode of entry into the body and almost all inhaled lead is absorbed whereas 20-70% of ingested lead is absorbed. The absorption of lead and its fate in the body mainly depends on physiologic characteristics of the exposed person including nutritional status, health and age (Ali, 1997).

The mean blood lead values of 41.36±2.71µg/dl observed for petrol station attendants in the present work compares with 42.50µg/dl reported by Reima et al. (1991) and 37.73µg/dl determined by Kentner et al. (1994) in their study of lead exposed workers in 2 battery factories in Germany. It compares however with 42.40µg/dl observed by Ademuyiwa et al. (2002) in their study on 57 male petrol station attendants in Abeokuta, Nigeria and is lower than the 70.6 µg/dl reported by Olubajo et al. (1992) in their work on male petrol station attendants in Lagos City.

Table (5): Demographic characterization of 115 subjects.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>N</th>
<th>Blood Lead Concentration (µg/dl)</th>
<th>Number of SH group in Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>Petrol Attendants</td>
<td>University Students</td>
<td>Petrol Attendants</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>15</td>
<td>17</td>
<td>22.56±4.21</td>
</tr>
<tr>
<td>26 – 30</td>
<td>38</td>
<td>13</td>
<td>46.34±2.07</td>
</tr>
<tr>
<td>31 – 35</td>
<td>20</td>
<td>5</td>
<td>57.44±6.52</td>
</tr>
<tr>
<td>36 – 40</td>
<td>7</td>
<td>-</td>
<td>55.67±3.05</td>
</tr>
<tr>
<td>Years of Occupational Lead Exposure</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>23</td>
<td>-</td>
<td>27.31±2.54</td>
</tr>
<tr>
<td>6 – 10</td>
<td>28</td>
<td>-</td>
<td>44.15±1.63</td>
</tr>
<tr>
<td>11 – 15</td>
<td>17</td>
<td>-</td>
<td>50.63±2.00</td>
</tr>
<tr>
<td>16 – 20</td>
<td>9</td>
<td>-</td>
<td>36.71±1.68</td>
</tr>
<tr>
<td>21 – 30</td>
<td>3</td>
<td>-</td>
<td>55.81±4.17</td>
</tr>
<tr>
<td>Smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>5</td>
<td>50.46±5.01</td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>30</td>
<td>36.37±2.46</td>
</tr>
<tr>
<td>Drinking</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>13</td>
<td>41.56±3.56</td>
</tr>
<tr>
<td>No</td>
<td>48</td>
<td>22</td>
<td>37.45±4.11</td>
</tr>
<tr>
<td>Educational</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Illiterate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Primary School</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Middle School</td>
<td>42</td>
<td>-</td>
<td>46.30±5.02</td>
</tr>
<tr>
<td>High School and above</td>
<td>38</td>
<td>35</td>
<td>37.21±3.65</td>
</tr>
</tbody>
</table>

N = Number of Students. Values are expressed in mean ± SD.
In the present investigation on the mean blood lead level was found to be 14.31±2.16 µg/dl among male undergraduate students of University of Agriculture located in Abeokuta, Nigeria. Ogunsola et al. (1994) found an average blood lead for non occupationally exposed adults in Lagos and Ile to be 13 µg/dl, Kapu et al. (1989) found a mean blood lead level of 6 µg/dl in their study on non-smoking male undergraduates of a University in Zaria city of Northern Nigeria. Olubajo et al. (1992) in their study found a mean blood lead level of 25.6 µg/dl among male undergraduate students of a University in Lagos city Nigeria, while Omokhodion (1994) reported 12 µg/dl average blood lead in adult population of Ibadan, another city in Nigeria Ademuyiwa et al. (2002) also found a mean blood lead level of 16.27 µg/dl among male undergraduate students of a University located in Abeokuta which is another city in Nigeria, which suggests that blood lead levels vary from one city to another as a result of the difference in the commercial activities and the number of industries located in these cities.

The present study shows that lead absorption caused slight increase of blood pressure. This is in close agreement with previous studies (Schwartz et al., 2001; Lee et al., 2000).

The present study demonstrates a pervasive elevation of blood lead level in petrol station attendants in Abeokuta, Nigeria and that atmospheric lead represents a significant source of human exposure to environmental pollutant in any urban areas. It is evident that factors like age, smoking status, drinking habits, education and years of occupational lead exposure has an influence on the concentration of lead accumulated in the body.

ACKNOWLEDGMENTS

The authors are grateful to our students, the proprietors of the various petrol stations and also the petrol sation attendants for their cooperation in this study. Our thanks are also due to Mrs. Adebawa of Biochemistry laboratory, University of Agriculture, Abeokuta, and Mr. M. Akanbi of Chemistry /Biochemistry department, Bowen University, Iwo, Nigeria for their technical support.

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Received July 20, 2008
Accepted March 10, 2009