The Effects of Lead on Some Markers of Liver and Kidney Functions of Lead Recycling Factory Workers are Mediated Through Increased Oxidative Stress

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Abstract

Background: Lead is a highly toxic metal of great public health importance.

Objectives: This cross-sectional study was conducted to evaluate the kidney and liver function status and the markers of oxidative stress among lead recycling factory workers in Anambra State, Nigeria.

Methods: A total of 82 subjects (41 experimental and 41 control subjects) aged 20-60 years were recruited in this study. Lead levels in whole blood were measured using atomic absorption spectrometry. Serum malondialdehyde (MDA) and uric acid levels as well as the activities of glutathione S-transferase (GST), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyltransferase (γ-GT) were measured using the spectrophotometric method. Superoxide dismutase (SOD) and catalase activities were determined using colorimetric method and Berthelot’s and Jaffe slot methods were used to measure urea and creatinine levels, respectively.

Results: The mean activities of SOD, GST, and catalase as well as estimated glomerular filtration rate (eGFR) were significantly lower in lead recycling workers compared with control group (P<0.05), whereas the mean activities of ALT, AST, ALP, and γ-GT, as well as blood lead, MDA, urea, creatinine, and uric acid levels significantly increased in lead recycling workers (P<0.05). Furthermore, blood lead level was found positively correlated with MDA and creatinine levels but negatively with eGFR and GST levels (P<0.05). MDA level showed positive and negative correlations with creatinine and eGFR (P<0.05), respectively.

Conclusion: This study revealed significant alterations in the levels of some biochemical and oxidative stress parameters in liver and kidney in lead recycling factory workers and showed a possible link between oxidative stress and the toxic effects of lead on the kidney and liver.

Keywords: Lead, Antioxidants, Oxidative stress, Kidney, Liver, Enzymes

Background

Lead, whether ingested or inhaled, is a highly toxic metal that affects practically all body organs and systems (1). In Nigeria, there are numerous small-scale and medium-scale industries and occupational workers (auto-mechanics, electricians, welders, painters, panel beaters, and the like) that use lead-based materials. Some of these industries have no workplace regulations for lead exposure. However, the extensive use of lead has led to serious public health issues, exposure to humans, and environmental damage in many regions of the world (2–4). Workers are typically exposed to lead by the inhalation of lead particles produced by burning lead-containing products during unofficial recycling, melting, stripping leaded paint, and using leaded gasoline (5).

The health consequences of lead exposure do not
Materials and Methods

Study Sites
This research was conducted in one of the lead recycling factories in Anambra State, Nigeria. Analyses of biochemical parameters were conducted at the facilities of the Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi.

Study Population
This cross-sectional study involved a total of 82 subjects which were divided into two equal groups, namely, experiment and control groups. The experimental group comprises 41 male lead-recycling workers aged 20-60 years who gave their written informed consent. A total population sampling method was employed, and only the subjects that did not meet inclusion criteria or did not give written informed consent were excluded from the study. These subjects have actively worked for 1-20 years in one of the lead recycling factories. Their work involved melting used battery cells using high-temperature furnace to remove impurities and then remolding them into lead bars which will be used in manufacturing new batteries. Likewise, the control group comprises 41 male subjects with the same age range as the experimental group. They were civil servants whose occupations do not expose them to lead. For comparative purposes, they resided in the same domestic vicinity of the experimental subjects. The purpose of matching them in terms of residential area was to cancel out other sources of lead exposures such as water, inhalation of exhaust fume, and the like, thus differentiating them only based on their occupation.

Inclusion Criteria
Both apparently healthy experimental male individuals who were occupationally exposed to lead and apparently healthy control male subjects who were not occupationally exposed to lead were recruited into the study with written informed consent.

Exclusion Criteria
The study excluded those who had a history of chronic conditions, including diabetes mellitus, cardiovascular disease, kidney disease, and the like as well as those who were taking any medications such as vitamins or mineral supplements that could affect the results.

Sample Collection
Six milliliters of blood sample were collected from each subject after 10-12 hours of overnight fasting, and four milliliters were dispensed into a sterile plain container and allowed to clot and retracted. The blood was centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then kept at -20°C for the analysis of all the biochemical and oxidative parameters. The remaining two milliliters of whole blood were dispensed into an ethylenediamine tetraacetic acid container for the determination of blood lead level.

Laboratory Methods
Superoxide dismutase (SOD) was assayed by the colorimetric method of Misra and Fridovich (24), while catalase activity was determined by the colorimetric method of Sinha (25). Further, glutathione-s-transferase (GST) activity and MDA were determined...
spectrophotometrically as described by Habig et al (26) and Gutteridge and Wilkins (27), respectively. Atomic absorption spectrometry was used to measure the amount of lead in whole blood according to the Hessel method (28). The AST and ALT activities were estimated using the spectrophotometric method by Bergmeyer et al (29), while ALP activity was measured using the spectrophotometric method by Schlebusch et al (30). In addition, γ-GT was determined using the spectrophotometric method of Persijn and van der Slik (31), and serum urea was determined by Berthelot’s method described by Kassirer (32) using a commercial kit from (Randox, UK). Moreover, serum creatinine was determined by Jaffe’s method as described by Lanon (33) using a commercial kit from (Randox, UK), while uric acid was determined via the spectrophotometric method described by Kageyama (34).

**Statistical Analysis**

For statistical analysis, the Statistical Package for Social Sciences (SPSS) version 21 was utilized. In terms of the variables, mean and standard deviation were used. The significance of the mean difference between the two independent variables was determined using an independent student t test. To evaluate the degree of relationship between two independent groups, Pearson’s correlation test was used, and the significance level was set at P<0.05.

**Results**

The mean level of blood lead and MDA significantly increased in lead recycling workers than in control subjects (P<0.001). However, the mean serum activities of SOD, GST, and catalase significantly decreased in lead recycling workers than in the control group (P<0.001), respectively (See Table 1).

When compared to the control group, the mean levels of urea, creatinine, and uric acid were significantly higher in the lead recycling workers (P<0.001) in each case; however, the mean level of estimated glomerular filtration rate (eGFR) was significantly lower (P<0.001) in the lead recycling workers, as depicted in Table 2. When compared to the control group, the mean serum activities of the liver enzymes (ALT, AST, ALP, and GGT) were significantly higher in the workers at the battery recycling factory (P<0.05), as illustrated in Table 3.

The blood lead levels correlated negatively with eGFR and GST in lead recycling workers (P<0.05). However, blood lead levels in lead recycling workers correlated positively with their MDA and creatinine levels (P<0.001). In addition, MDA correlated positively with creatinine levels but negatively with eGFR in lead recycling workers (P<0.001), as seen in Table 4.

**Discussion**

In this study, workers who recycled lead had higher blood lead levels. This result supports the idea that occupational exposure raises blood lead levels in exposed people. The important statistical finding of a higher blood lead level in this study is consistent with the numerous reports of related investigations (13,14). The results revealed significant decreases in the serum activities of SOD, catalase, and GST as well as a significant increase in the serum level of MDA in the lead-exposed group. These findings suggested increased oxidative stress in lead-exposed individuals.

In line with this study, Soltaninejad et al (35) as well as Vaziri et al (36) reported a decrease in the activities of some enzymatic antioxidants involved in defense against oxidative stress such as SOD and catalase. Haleaghrara et al also reported a decrease in the activity of GST in lead-exposed group (37). Several other studies carried out on lead-exposed animals also found an increase in lipid peroxidation or a decrease in antioxidant defense mechanism (38, 39).

In this study, levels of serum creatinine, urea, and uric acid significantly increased, while the level of estimated creatinine-based GFR decreased in individuals involved in lead recycling. Additionally, there were significant positive correlations between the levels of blood lead and serum creatinine as well as MDA and serum creatinine.

Raised serum ALT and AST activities which are indicators of hepatocellular damage and raised serum ALP and GGT activities which are indicators of hepatobiliary damage were observed in the lead recycling workers in the current study. This could be due to the lead accumulation in the liver and its ability to exert oxidative damage to hepatic cell membranes causing the leakage of these enzymes into the circulation (26). The results of this investigation were consistent with those of Dongre et al who found that automobile employees had higher transaminase (ALT and AST), ALP, and GGT activity compared to control subjects (37).

However, the present work also demonstrated a significant inverse relationship between blood lead levels and estimated GFR as well as between MDA and eGFR. These findings point to the fact that there exists a link between oxidative stress and the effects of lead poisoning on the kidney. This is consistent with other research that claims lead-induced oxidative stress is one of the major contributory elements to the development of lead toxicity (40).

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**Table 1. Blood Lead Level, Serum MDA, and Some Enzymatic Anti-oxidants in Lead Recycling Workers and Control Subjects (Mean ±SD)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lead Recycling Workers (n=41)</th>
<th>Control (n=41)</th>
<th>t-value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (µg/dL)</td>
<td>46.27 ± 11.51</td>
<td>15.88 ± 6.82</td>
<td>14.542</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>5.17 ± 1.08</td>
<td>3.49 ± 0.82</td>
<td>8.875</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>2.05 ± 0.39</td>
<td>2.87 ± 0.72</td>
<td>-6.431</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GST (U/L)</td>
<td>4.30 ± 1.36</td>
<td>6.58 ± 2.43</td>
<td>-5.220</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Catalase (U/mL)</td>
<td>11.85 ± 2.38</td>
<td>20.04 ± 5.28</td>
<td>-9.057</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Significant.
Table 2. Serum Levels of Kidney Markers in Lead Recycling Workers and Control Groups (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lead Recycling Workers (n = 41)</th>
<th>Control (n = 41)</th>
<th>t-value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mmol/L)</td>
<td>4.70 ± 0.83</td>
<td>3.30 ± 0.77</td>
<td>7.972</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>98.02 ± 16.91</td>
<td>85.17 ± 12.70</td>
<td>3.892</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>81.43 ± 19.89</td>
<td>120.48 ± 20.01</td>
<td>-8.862</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum uric acid (μmol/L)</td>
<td>321.50 ± 73.06</td>
<td>257.31 ± 54.69</td>
<td>4.504</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Significant.

Table 3. Serum Liver Enzymes in Lead Recycling Workers and Control Groups (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lead Recycling Workers (n = 41)</th>
<th>Control (n = 41)</th>
<th>t-value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>18.67 ± 9.25</td>
<td>8.62 ± 4.28</td>
<td>6.311</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>35.61 ± 11.21</td>
<td>19.37 ± 7.63</td>
<td>7.670</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>93.97 ± 20.73</td>
<td>78.91 ± 19.79</td>
<td>3.365</td>
<td>0.001*</td>
</tr>
<tr>
<td>γ-GT (U/L)</td>
<td>36.48 ± 29.41</td>
<td>25.42 ± 8.28</td>
<td>2.318</td>
<td>0.023*</td>
</tr>
</tbody>
</table>


* Significant.

Table 4. The Correlation between Blood Lead levels and Creatinine, eGFR, MDA, and GST in Lead Recycling Workers and Control Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lead Recycling Workers (r)</th>
<th>Control (r)</th>
<th>P-value</th>
<th>Lead (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lead vs. creatinine</td>
<td>0.666</td>
<td>0.058</td>
<td>0.731</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead vs. eGFR</td>
<td>-0.327</td>
<td>-0.108</td>
<td>0.531</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead vs. MDA</td>
<td>0.540</td>
<td>0.391</td>
<td>0.019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead vs. GST</td>
<td>-0.316</td>
<td>-0.052</td>
<td>0.767</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA vs. creatinine</td>
<td>0.556</td>
<td>-0.076</td>
<td>0.636</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA vs. eGFR</td>
<td>-0.546</td>
<td>0.006</td>
<td>0.970</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: eGFR: Estimated glomerular filtration rate; MDA: Malondialdehyde; GST: Glutathione-s-transferase.

* Significant.

Conclusion
This study revealed significant alterations in the levels of some biochemical parameters of the liver, kidney, and oxidative stress in lead recycling factory workers. The harmful effects of lead on the kidney and liver may, however, be related to MDA (a marker of oxidative stress) levels.

Authors’ Contribution
Conceptualization: Christian Ejike Onah, Chukwuemeka Samuel Meludu, Chudi Emmanuel Dioka.
Data curation: Christian Ejike Onah, Chukwuemeka Samuel Meludu.
Formal Analysis: Christian Ejike Onah, Chukwuemeka Emmanuel Ogbodo
Project administration: Christian Ejike Onah, Chukwuemeka Samuel Meludu, Chudi Emmanuel Dioka.

Software: Christian Ejike Onah, Chukwuemeka Emmanuel Ogbodo.
Supervision: Chukwuemeka Samuel Meludu and Chudi Emmanuel Dioka.
Visualization: Christian Ejike Onah, Chukwuemeka Emmanuel Ogbodo.
Writing–review & editing: Chukwuemeka Emmanuel Ogbodo, Stephen Monday Suru, Chukwuemeka Samuel Meludu, Chudi Emmanuel Dioka.

Competing Interests
None.

Ethical Approval
The Ethics Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus granted their clearance for this study with the approval number FBMS/EC/004/2014.

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