Assessment of liver functions in occupationally exposed subjects working in lead acid battery factory in Nnewi

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Citation
Okpogba Aloysius Ngozyka, Ogbodo Emmanuel Chukwuemeka, Agada Uchenna Nelson, Nkama Venite Isioma, Akabuogu Roseline Kwenyerechi, Amah Akuma Kalu, Okwara Nuratu Adejumoke, Odumodu Ifeakandu Onyedikachukwu. Assessment of liver functions in occupationally exposed subjects working in lead acid battery factory in Nnewi. Medical Science, 2020, 24(106), 4821-4829

ABSTRACT
Lead (Pb) is an important environmental pollutant causing significant public health problems in many parts of the world and may be implicated in the pathology of the liver. This study was designed for the assessment of liver functions in lead acid battery factory workers occupationally exposed to hazardous effects of lead in their workplace in Nnewi. A total of 78 apparently healthy individuals aged between 18 and 56 years were recruited for the study. Thereafter, 6ml of venous blood sample was collected from each individual into plain container for the evaluation of biochemical indices. Total protein (TP), Albumin (ALB), Total bilirubin (TB), direct bilirubin (DB), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase activity (ALP) were assayed using established standard laboratory methods. Results revealed no significant alterations in the levels of serum ALB, TP, DB,
AST and ALT (p>0.05) with significantly reduced levels of TB and ALP activity among the lead acid battery factory workers (P<0.05) than in control subjects which suggest no underlying liver pathology as a result of occupational exposure.

Keywords: Heavy metals, Lead, Liver, Liver functions, Age, LOS, Gender.

1. INTRODUCTION

Lead (Pb) is an environmental pollutant, especially due to its anthropogenic activity (Humayun et al., 2015). Exposure to Pb is known to cause harmful effects of varying degree because of its ability to bio-accumulate (Humayun et al., 2015). The widespread use of lead has resulted in extensive environmental contamination, human exposure and significant public health problems in many parts of the world (World Health Organization (WHO), 2013a). Exposures to lead have been shown to play key roles in the pathology of various organs and systems in the human body (Lamas et al., 2016; Okpogba et al., 2020), although some studies noted no significant effects on some principal organs in humans occupationally exposed (Okpogba et al., 2020; Okpogba et al., 2020). Expectedly, heavy metal contamination has been documented in the present study area previously by several authorities especially lead contamination (Okpogba et al., 2020). Numerous studies have shown elevated levels of lead among occupationally exposed persons across the globe (Okpogba et al., 2020). Importantly, significant elevation in serum alanine transaminase (ALT) and alkaline phosphatase (ALP) activities with no significant alteration in serum aspartate transaminase (AST) in battery manufacturing workers has been recorded in the past (Al-Mzaien et al., 2019).

Several other similar studies also showed similar findings (El-Shafei, 2011; Rahul et al., 2017; Ataro et al., 2019), whereas some studies indicated lack of significant differences in ALT, AST and ALP activities between the automobile workers and control (Adejumo et al., 2016). Furthermore previous reports have shown no significant alterations in the levels of bilirubin and albumin (ALB) (Adejumo et al., 2016; Al-Mzaen et al., 2019) with a corresponding significant statistical increase in total protein levels among factory workers or occupationally exposed individuals (Ataro et al., 2019). The differing results documented in literature regarding the integrity of the liver among occupationally exposed persons perhaps is a pointer to the need for further studies in this respect. Thus, our aim was to carry out an assessment of liver functions in occupationally exposed subjects working in lead acid battery factory in Nnewi Nigeria.

2. MATERIALS AND METHODS

Study Design and subjects

This study is a cross-sectional study designed for the assessment of liver functions in occupationally exposed subjects working in lead acid battery factory in Nnewi. A total of 39 apparently healthy individuals in the exposed group (lead acid battery manufacturing factory workers) aged between 19 and 56 years were recruited for the study. The exposed group comprised workers from lead acid battery manufacturing factories who were constantly being exposed to effluents from lead acid battery manufacturing factory. The control groups were made up of thirty-nine (39) staff and undergraduate students of Nnamdi Azikiwe University, that resides about 5-10 km away from the factory sites aged between 18 and 44 years. Thereafter, 6ml of venous blood sample was collected from each individual into plain container for the evaluation of biochemical indices.

Inclusion Criteria

Lead factory workers without any known disease condition aged between 19 and 56 years and control individual (non-exposed groups) were included in this study

Exclusion Criteria

Individuals of any known kidney disease, liver disorder, alcoholics and smokers as well as those outside the age limits were excluded from the study.

Methods

Determination of Serum Aspartate Aminotransferase (AST) Activity

Aspartate Aminotransferase (AST) Activity was determined using the colorimetric method of Rietman and Frankel (1957).

Determination of Serum Alanine Aminotransferase (ALT) Activity

Alanine aminotransferase activity (ALT) was estimated according to the method of Rietman and Frankel (1957).

Determination of Serum Alkaline Phosphatase Activity
Alkaline phosphatase activity (ALP) was assayed according to the method as described by Mauro and Renze (2013).

**Determination of Serum Conjugated (Direct bilirubin) and Total Bilirubin levels**

Estimation of Bilirubin level was done using the colorimetric method described by Jendrassik and Grof (1938).

**Serum total protein (TP) Assay**

The total protein concentration was determined by Biuret method which depends on the presence of peptide bonds in proteins that react with Cu$^{2+}$ ions in alkaline solutions to form a coloured product, whose absorbance is measured spectrophotometrically at 540nm wavelength.

**Determination of albumin**

The albumin concentration in the serum was determined following the method of Doumas and Watson (1971).

**Statistical Analysis**

The data obtained were analyzed for both control and test group by Students t-test and Pearson’s bivariate correlation coefficient using Statistical package for social sciences (SPSS) (Version 16) software and statistical significance was pecked at P<0.05.

### 3. RESULTS

The liver function status of factory workers and control subjects is presented in Table 1. The total protein (TP) values of lead acid battery factory V (7.41±0.13) workers were not statistically different (p>0.05) from the control (7.82±0.06). The mean albumin (Alb) level were similar when compared between control and test subjects (p>0.05). Total bilirubin (TB) was significantly declined (p<0.05) in the factory subjects when compared with the control. On the other hand, mean (±SEM) DB values of the control subjects (7.68±0.52) was non-significantly elevated (p>0.05) in factory V (7.92±0.45), workers. The ALT and AST levels of control subjects and workers of lead acid battery did not differ significantly when compared between both groups (p>0.05). ALP activities of control subjects (77.41±1.52) was significantly reduced (p<0.05) in factory V (26.87±1.11).

### Table 1 Liver function status of the factory V workers

<table>
<thead>
<tr>
<th></th>
<th>TP (g/dL)</th>
<th>Alb (g/dL)</th>
<th>TB (µmol/L)</th>
<th>DB (µmmol/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr N (n=39)</td>
<td>7.82±0.06abc</td>
<td>4.32±0.07b</td>
<td>18.28±0.96d</td>
<td>7.68±0.52b</td>
<td>11.61±0.91a</td>
<td>7.92±0.83a</td>
<td>77.41±1.52b</td>
</tr>
<tr>
<td>V (n=38)</td>
<td>7.41±0.13ab</td>
<td>4.89±0.08bc</td>
<td>14.54±0.44c</td>
<td>7.92±0.45b</td>
<td>9.62±0.51a</td>
<td>7.34±0.59a</td>
<td>26.87±1.11a</td>
</tr>
</tbody>
</table>

Values are in mean±SEM; within the column, mean with different superscript letters are statistically significant (p<0.05).

Key: Contr N: Control subjects from Nnewi; V: Factory workers from Lead acid battery factory; TP: Total Protein; Alb: Albumin; TB: Total Bilirubin; DB: Direct bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase

Table 2 presents the analyses of the classification of liver function status of factory V workers according to age while Table 3 presents the effect by LOS. Regression of the liver function status with age is presented in Figure 1 while regression with LOS is in Figure 2. In order to determine the degree of relationship between the liver function status of factory V workers and age and LOS, correlation analyses was performed. TP, TB, DB, AST and ALP were positively correlated while ALB and ALT were negatively correlated with age. Except for ALB, AST, ALT and ALP that were positively correlated, TP, TB and DB were negatively correlated with LOS. Similar analyses with LOS also revealed no significant effect (p>0.05) indicating that both age and LOS had no effect on the liver function parameters of factory V workers in this study.

The classification of liver function status of factory workers according to gender is presented in Table 4. Further observation shows that the TP level of factory V was non-significantly reduced when compared with the control subjects (p>0.05) while the same trend was obtained in the female factory workers of the factory V when compared with the control. While the Alb concentrations of the male factory workers were elevated against the control subjects, though non-significantly (p>0.05). TB levels in all the male factory workers reduced significantly when compared with the control (p<0.05), however, that of the females did not differ significantly with the control (p>0.05). The DB values of male factory V workers were elevated but non-significantly (p>0.05). In the female category, the DB levels of factory V workers were significantly elevated (p<0.05) than in control subjects. There were reduced
AST levels in males of factory V workers (p<0.05) when compared with the control. The AST levels in the females followed the same trend in the males. There was no significant difference between the ALT levels of the males and female factory workers (p>0.05) when compared with the control. ALP levels in the male and female factory V workers (p<0.05) were significantly increased than in control.

**Table 2** Effect of age on the liver function status of battery factory (V) workers.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>TP (g/dL)</th>
<th>ALB (g/dL)</th>
<th>TB (µmol/L)</th>
<th>DB (µmol/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control N (n=39)</td>
<td>7.82±0.06a</td>
<td>4.32±0.07a</td>
<td>18.28±0.96a</td>
<td>7.68±0.52a</td>
<td>11.61±0.91a</td>
<td>7.92±0.83a</td>
<td>77.41±1.52b</td>
</tr>
<tr>
<td>18-30yrs (n=25)</td>
<td>7.38±0.17a</td>
<td>4.88±0.10a</td>
<td>14.57±0.53a</td>
<td>7.85±0.52a</td>
<td>9.88±0.67a</td>
<td>7.56±0.70a</td>
<td>27.08±1.48a</td>
</tr>
<tr>
<td>31-40yrs (n=7)</td>
<td>7.40±0.26a</td>
<td>4.91±0.25a</td>
<td>13.92±1.26a</td>
<td>7.36±1.13a</td>
<td>8.86±0.86a</td>
<td>7.57±1.74a</td>
<td>23.91±2.50a</td>
</tr>
<tr>
<td>41-50yrs (n=4)</td>
<td>7.50±0.20a</td>
<td>4.90±0.12a</td>
<td>14.96±0.82a</td>
<td>8.55±0.98a</td>
<td>9.75±1.89a</td>
<td>7.00±1.91a</td>
<td>28.18±1.01a</td>
</tr>
<tr>
<td>51-60yrs (n=2)</td>
<td>7.60±0.80a</td>
<td>4.88±0.02a</td>
<td>15.39±3.42a</td>
<td>9.40±5.00a</td>
<td>9.00±2.00a</td>
<td>4.50±0.50a</td>
<td>31.95±2.45a</td>
</tr>
</tbody>
</table>

Values in mean±SEM
Within column, means with different superscript letter alphabets are statistically significant (p<0.05)

KEY: TP= Total Protein; ALB=Albumin; TB=Total Bilirubin; DB=Direct Bilirubin; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline phosphatase.

**Table 3** Effect of LOS on liver function status of factory V workers

<table>
<thead>
<tr>
<th>LOS RANGE</th>
<th>TP (g/dL)</th>
<th>ALB (g/dL)</th>
<th>TB (µmol/L)</th>
<th>DB (µmol/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control N (n=39)</td>
<td>7.82±0.06b</td>
<td>4.32±0.07a</td>
<td>18.28±0.96a</td>
<td>7.68±0.52a</td>
<td>11.61±0.91a</td>
<td>7.92±0.83a</td>
<td>77.41±1.52b</td>
</tr>
<tr>
<td>0-5yrs (n=12)</td>
<td>7.50±0.19ab</td>
<td>4.79±1.21b</td>
<td>14.11±1.01a</td>
<td>8.21±0.96a</td>
<td>8.36±0.75a</td>
<td>6.25±1.20a</td>
<td>26.52±1.96a</td>
</tr>
<tr>
<td>6-10yrs (n=19)</td>
<td>7.44±0.21ab</td>
<td>4.79±0.11b</td>
<td>14.89±0.56a</td>
<td>8.01±0.62a</td>
<td>10.59±0.87a</td>
<td>8.00±0.85a</td>
<td>26.85±1.76a</td>
</tr>
<tr>
<td>11-15yrs (n=2)</td>
<td>7.206±0.32a</td>
<td>5.45±0.35c</td>
<td>15.39±1.21a</td>
<td>8.96±1.02a</td>
<td>9.75±1.25a</td>
<td>7.00±1.91a</td>
<td>27.33±1.54a</td>
</tr>
<tr>
<td>16-20yrs (n=5)</td>
<td>7.40±0.34ab</td>
<td>5.02±0.10ab</td>
<td>13.68±1.08a</td>
<td>6.02±1.14a</td>
<td>9.00±0.84a</td>
<td>8.00±1.26a</td>
<td>27.42±4.11a</td>
</tr>
</tbody>
</table>

Values are in mean±SEM;
Within the same column, means with different letters are statistically significant (p<0.05)

LOS=Length of service; TP=Total Protein; ALB=Albumin; TB=Total Bilirubin; DB=Direct Bilirubin; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline Phosphatase.
Figure 1 Regression of liver function status of factory V workers with age
Figure 2 Regression of liver function status of factory V workers with LOS.
important cardiac muscles, therefore the serum concentrations of these similar findings differed with our present ion. These enzymes, in consequence, leak out of the ents of the factory. Serum AST and ry was not enough to induce cytotoxic effects that can result in kers.

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ificant change in the concentration of these enzymes. appreciable changes in the concentration of these enzymes. Factory workers seem to suggest that heavy metal levels in this facto

as a significant role of the liver that is not compromised. In a related study by Dioka et al (2004) on the liver and renal function tests on Pb of Nnewi mechanics and artisans, they reported no significant change in the total protein and albumin levels of the tested individuals even when Pb level was significantly elevated in the exposed group suggesting that Pb had no effect on the synthetic role of the liver. However, some reports have recorded significant increase in total protein level among occupationally exposed people compared with non-exposed group (Ate

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tables under study. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are enzymes produced by the liver tissues

Dioka Mzaien (2004) which showed no apparent change in the total bilirubin concentrations of artisans occupationally exposed to

This study assessed the liver function status of the acid battery manufacturing factory workers in Nnewi. The liver is an important organ for the synthesis of proteins, cholesterol, TAGs and lipoprotein complexes. The results of the total protein concentration of the control subjects used in this study did not vary statistically from those of the acid battery factory workers. Also, no significant changes were observed between the albumin concentrations of the control subjects and factory workers in the lead acid battery manufacturing factory. This result shows that the synthetic role of the liver has not been compromised. In a related work by Dioka et al (2004) on the liver and renal function tests on Pb of Nnewi mechanics and artisans, they reported no significant change in the total protein and albumin levels of the tested individuals even when Pb level was significantly elevated in the exposed group suggesting that Pb had no effect on the synthetic role of the liver. However, some reports have recorded signif

Total protein and albumin levels of the tested individuals even when Pb level was significantly elevated in the exposed group

Decreased total bilirubin concentrations were observed in the factory workers compared with the control subjects. Bilirubin is the main pigment formed from heme degradation. There were significant differences between the total bilirubin levels of the control subjects and the factory workers, however, the values fell within the normal bilirubin range. The difference might be due to over activity of the liver of the control subjects being very young individuals and involved in active work. The result of decreased bilirubin concentrations in the factory workers indicates that there is no enhanced haemoglobin degradation going on in the factory workers. This is confirmed by the normal haemoglobin concentrations observed in these groups of workers. This result is similar to the report of Dioka et al (2004) which showed no apparent change in the total bilirubin concentrations of artisans occupationally exposed to lead in mechanic village in Nnewi. Other similar studies corroborates with the current findings (Adejumo, et al., 2016; Al-Mzaien, et al., 2019).

To fully determine the state of the cellular integrity of the liver cells of the factory workers in this study, there is the need to assess the serum non-secretor, non-specific enzymes which are native to the liver tissues under study. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are enzymes produced by the liver tissues- the hepatocytes. The levels of these enzymes are usually increased in blood in conditions in which the hepatocytes are damaged. These enzymes, in consequence, leak out of the liver into the blood stream. Conditions that may cause this include hepatitis due to viral attack, drug and alcohol intoxication. Another condition that can cause this is liver cell death resulting from other cases such as shock.

The results of this study showed that the specific activities of AST and ALT were within the serum normal range in the lead acid battery factory and control. This is an indication that there was no cytotoxic effect from the effluents of the factory. Serum AST and ALT were elevated in artisans than control in a work reported by Dioka et al. (2004) while these same enzymes were elevated in gas station workers while only ALT was elevated in taxi drivers (Kapaki et al., 1998). Other similar findings differed with our present results (Al-Mzaien et al., 2019; Ataro et al., 2019; Rahul et al., 2017). It is noteworthy that the serum activity of these enzymes is not solely accounted for by the liver but also by the muscles especially the cardiac muscles, therefore the serum concentrations of these enzymes depend on the health status of both the liver and the muscles. The general pattern of these results in the acid battery factory workers seem to suggest that heavy metal levels in this factory was not enough to induce cytotoxic effects that can result in appreciable changes in the concentration of these enzymes.

In this study, reduced serum alkaline phosphatase (ALP) activity was observed in the acid battery manufacturing factory workers when compared with their serum levels in the control subjects. However, the work of Orisakwe et al., (2007) on the AST, ALT and ALP

<table>
<thead>
<tr>
<th>FACTORY</th>
<th>SEX</th>
<th>TP (g/dL)</th>
<th>ALB (g/dL)</th>
<th>TB (µmol/L)</th>
<th>DB (µmol/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(n=29)</td>
<td>M</td>
<td>7.88±0.06b</td>
<td>4.38±0.08abcd</td>
<td>18.14±0.91c</td>
<td>7.62±0.62b</td>
<td>11.66±1.08a</td>
<td>8.24±0.95a</td>
<td>78.03±1.74a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.10±0.06a</td>
<td>4.16±0.15abc</td>
<td>18.70±2.80c</td>
<td>7.86±0.98b</td>
<td>11.50±1.76a</td>
<td>7.00±1.74a</td>
<td>75.60±3.20a</td>
</tr>
<tr>
<td>V(n=27)</td>
<td>M</td>
<td>7.40±0.14d</td>
<td>4.90±0.76cd</td>
<td>14.38±0.47bc</td>
<td>7.92±0.52bc</td>
<td>9.77±0.67d</td>
<td>7.59±0.75p</td>
<td>26.93±1.32cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.43±0.28a</td>
<td>4.86±0.21cd</td>
<td>14.92±1.03bc</td>
<td>7.88±0.95b</td>
<td>9.27±0.61a</td>
<td>6.73±0.86p</td>
<td>26.71±2.13a</td>
</tr>
</tbody>
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Within column, means with different superscript letter alphabets are statistically significant (p<0.05)

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**4. DISCUSSION**

This study assessed the liver function status of the acid battery manufacturing factory workers in Nnewi. The liver is an important organ for the synthesis of proteins, cholesterol, TAGs and lipoprotein complexes. The results of the total protein concentration of the control subjects used in this study did not vary statistically from those of the acid battery factory workers. Also, no significant changes were observed between the albumin concentrations of the control subjects and factory workers in the lead acid battery manufacturing factory. This result shows that the synthetic role of the liver has not been compromised. In a related work by Dioka et al (2004) on the liver and renal function tests on Pb of Nnewi mechanics and artisans, they reported no significant change in the total protein and albumin levels of the tested individuals even when Pb level was significantly elevated in the exposed group suggesting that Pb had no effect on the synthetic role of the liver. However, some reports have recorded significant increase in total protein level among occupationally exposed people compared with non-exposed group (Ataro et al., 2019).

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In this study, reduced serum alkaline phosphatase (ALP) activity was observed in the acid battery manufacturing factory workers when compared with their serum levels in the control subjects. However, the work of Orisakwe et al., (2007) on the AST, ALT and ALP
levels of paint factory workers in Nkpor Nigeria reported no significant differences between the levels of these enzymes in the exposed and unexposed workers even when they reported significant elevated serum lead, cadmium and nickel levels in the same subjects.

5. CONCLUSION
This study has shown no significant alterations in the levels of serum albumin, total protein, direct bilirubin, AST and ALT with significantly reduced levels of total bilirubin and alkaline phosphatase activity among the lead acid battery factory workers which is suggestive of no underlying liver pathology as a result of occupational exposure.

Acknowledgement
We thank the patients who were all participated in and contributed samples to the study.

Author Contributions
Okpogba Aloysius Ngozyka was the first author who elicited and generated the idea, started and supervised the work. Ogbodo Emmanuel Chukwuemeka is the corresponding author who contributed to sample analysis and wrote the manuscript. Agada Uchenna Nelson, Nkama Venite Isioma and Akabuogu Roseline Kwenyerechi contributed to participant’s recruitment, sample collection and laboratory analysis. Amah Akuma Kalu, Okwara Nuratu Adejumoke and Odumodu Ifeakandu Onyedikachukwu contributed to data analysis and proof read the manuscript.

Funding
This study has not received any external funding.

Conflict of Interest
The authors declare that there are no conflicts of interests.

Informed consent
Written and Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Ethical approval
The study was approved by the Medical Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (ethical approval code: NAUTH/CS/66/Vol.2/149).

Data and materials availability
All data associated with this study are present in the paper.

REFERENCES AND NOTES


Peer-review
External peer-review was done through double-blind method.