Modulation of renal function indices of the occupationally lead exposed Bangladeshi automobile workers

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ABSTRACT

Objectives

This study aims to compare serum uric acid (SUr) and renal function indices such as serum urea (SU), blood urea nitrogen (BUN), creatinine (SCr), urinary creatinine (UCr) and glomerular filtration rate (GFR) of the lead exposed automobile workers with healthy controls and evaluates association of serum lead (Pb-S) with renal function.

Methods

Of the total 70 male individuals included in this study, 45 were automobile workers and 25 were age and BMI matched healthy controls. For all the participants, levels of serum lead and their renal function indices were measured using standard assay methods.

Results

Automobile workers (n=45) had significantly higher (p=0.001) level of Pb-S (40.5±11.2 µg/dL) compared to age and BMI matched healthy controls (n=25, 29.7±10.1 µg/dL). Workers had lower levels of SU, SCr, BUN and UCr (p<0.01) but higher GFR (p=0.117) than controls. Both groups had similar levels of SUr (6.6±1.9 vs 6.9±1.1 mg/dL, respectively). Pb-S in workers demonstrated no relationship with any of the individual renal function indices. Linear regression analysis showed a positive relation of Pb-S of the workers with SCr and GFR, while BMI was negatively correlated. SUr showed a positive correlation with SCr and GFR in the workers while control subjects did not.

Conclusion

These data indicated modulation of renal function indices among the occupationally lead exposed Bangladeshi automobile workers.

Keywords: Automobile workers, lead toxicity, glomerular filtration rate, serum lead, renal dysfunction.

1. Introduction

Toxic metals such as lead, cadmium, and arsenic constitute significant potential threats to human health in both occupational and environmental settings (Hu, 2000). Acute or chronic lead exposure has been associated with renal function impairment and increased serum uric
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acid level (Perazalla, 1996; Lin et al. 2002; Lin et al. 2001; Crawswell et al., 1984; Colleoni and fAmico, 1986). Acute exposure to high concentrations of lead can result in proximal tubular damage with characteristic histologic features and manifested by glycosuria and aminoaciduria (Loghman-Adham, 1997). Study in individuals with low-level lead exposure has shown a positive correlation between blood lead (Pb-B) levels and serum creatinine or creatinine clearance (Loghman-Adham, 1997). Chronic low-level exposure to lead is associated with increased urinary excretion of low molecular weight proteins and lysosomal enzymes (Loghman-Adham, 1998). Lead showed adverse effect on renal function in older Korean workers assessed by blood urea nitrogen (BUN), serum creatinine and creatinine clearance as a measure of renal function (Jung et al., 1998). It was also reported that mild elevation of uric acid is associated with subclinical kidney disease in lead-exposed people (Johnson et al., 2003; Weaver et al., 2005). However, findings regarding renal function toxicity mediated by lead toxicity are incompatible. Levels of Pb-B in battery workers (with mean Pb-B of 53.5 µg/dl) in South Africa, showed a positive relationship with serum creatinine and uric acid, but had no effect on blood urea (Ehrlich et al., 1998). In contrast, Pb-B with an average value of 43 µg/DL in chronically exposed male workers did not affect the levels of serum creatinine and uric acid (Roels et al., 1994). Colleoni and fAmico (1986) reported association of chronic lead exposure with increased levels of serum creatinine and blood urea, but not with uric acid.

The air of Dhaka city holds 463 nanograms per cubic meter of lead - the highest in the world (IARC, 1980). Bangladeshi school-going children from different areas of Dhaka city and living in a close proximity to highway or intersection of the high way showed very high levels of Pb-B compared to the standard level set by Centers for Disease Control and Prevention, CDC (Wahed et al., 1999; Khan et al., 1999; Kaiser et al., 2001). However, there is no report regarding the status of lead in the body fluids of Bangladeshi adults who are more prone to lead exposure due to their occupation. Also, the effects of free fraction of serum lead on the levels of serum uric acid and renal function indices have not yet been conducted among Bangladeshi adults.

Thus, this study aims to (i) measure the levels of lead in the serum of automobile workers and healthy controls, (ii) compare serum lead (Pb-S) levels and serum uric acid, and such renal function indices as serum creatinine, urea, blood-urea nitrogen (BUN) and creatinine clearance of automobile workers with apparently healthy individuals from other occupation; (iii) find out relationship of these biochemical parameters with the levels of Pb-S.

2. Materials and method

2.1 Study subjects and sample collection

A total of 70 subjects (all males) were enrolled in this study. Of them, 45 were automobile workers who had to work daily for 8-12 hours. The remaining 25 were controls living in residential areas away from any nearby factories and they are businessmen, students, teachers and university office bearers. All the control subjects were interviewed for possible exposure to lead and none reported any. All of the garages visited did not have proper gas exhaust system and none of the workers were taking any protective measures to minimize health hazards from inhalation of toxic particles, especially lead and gasoline. The garages were involved in handling automobile fluids, recycling fluids that include petrol attendants and petrol refinery workers, car denting and painting, repairing radiators, welding and handling corrosion pipe filters. Blood samples were collected after getting full consent from each
participant with the help of an expert phlebotomist in presence of a medical practitioner. About 5 mL peripheral blood was collected at a single time period and then, centrifuged at 1500 g for 5 minutes. Serum samples were collected and stored at −70°C until further analysis. Subjects were randomly selected from those who were exposed for different periods to toxic automobile gas discharge and so more likely to develop associated complications. However, the inclusion criteria for the randomly selected subjects from the automobile and control groups included: (1) no evidence of prior or current use of nephrotoxic drugs, chronic medication for gout, EDTA chelation treatment, analgesic misuse, and herbal drugs; (2) no other nephrotoxic chemical exposure (i.e., cadmium and mercury); (4) no history of hypertension and diabetes mellitus; (3) more than two months of employment period.

2.2 Questionnaire

A questionnaire was prepared and all the subjects were fully aware about the importance of the study. The following information from all the participants was collected using a structured questionnaire: sociodemographic data, age, education, living areas within Dhaka city, occupational history, duration of work, health status and cigarette smoking. The participants were interviewed one-by-one to gather information about the questionnaires.

2.3 Determination of lead in serum

All measurements were carried out with a Perkin Elmer Analyst 800 Atomic Absorption Spectrometer equipped with a heated graphite furnace. Unspecific light absorption was corrected by continuum light source (Hollow cathode lamp) Zeeman background correction. The electrode less lamp for lead (Pb) from Perkin Elmer was operated at 10 mA with lamp energy of 65 Watt. The lead (Pb) wavelength used in this work was 283.3 nm. The volume of diluted sample and the calibration solutions pipetted into the graphite tube was 20 µL. The volume of chemical matrix modifiers [NH₄H₂PO₄ + Mg (NO₃)₂] in solution was 13 µL. Argon, 99.996% (White Martins, Belo Horizonte, MG, Brazil), was used as purge gas. Transverse seated graphite tubes with integrated platform (Perkin Elmer, Part Number B3001262 – B3001261) were used in all studies.

2.4 Determination of creatinine in serum and urine

Levels of creatinine in serum samples were measured based on the reaction of creatinine with sodium picrate. To measure creatinine, 100 µL of serum sample reacts with 1000 µL of alkaline picrate that forms red complex. The absorbance was measured at 492 nm and the time interval chosen for measurements that avoids interferences of other serum constituents. The intensity of the color was directly proportional to the concentration of serum creatinine which was expressed as mg/dL. Levels of creatinine in the spot urine samples were measured using same way.

2.5 Determination of glomerular filtration rate

Equations to estimate GFR are the most common method for assessing kidney function clinically and in large epidemiologic studies, where GFR assessment with an exogenous filtration marker is not possible. Estimated creatinine clearance as an index of Glomerular Filtration Rate (GFR) was calculated using the Cockcroft and Gault equation [equation (i)] (Kasiske and Keane, 2000) given below:

\[ C_{Cr} = \frac{(140-Age) \times \text{Body Mass}}{\text{Plasma creatinine} \times 72} \]  

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**Zinat, A, Hossain, M, Bhowmik, S, Khanom, M, Islam, L.N, Nabi AHMN**

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Where, $C_{Cr}$ indicates estimated creatinine clearance and age, body mass as well as plasma creatinine were considered in years, kilogram, mg/dL, respectively.

2.6 Determination of the levels of serum urea

Levels of serum urea in both automobile workers and control groups were determined by adding reagents to the serum samples according to the instruction of the reagent manufacturer and incubated for 15 minutes at $37^\circ$C. The intensity of the color was measured at 510 nm against the water as blank which was directly proportional to the levels of urea present in serum sample and the results were expressed as mg/dL.

2.7 Determination of the levels of uric acid in serum

Serum uric acid was determined using uricase that transformed uric acid into allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide formed colored complex which is proportional to the levels of serum uric acid. Serum sample (25 mL) was mixed with 1000 mL of reagent and then, incubated at $37^\circ$C for 5 minutes. Standard was prepared in the same way. Absorbance of the sample was taken at 520 nm against the reagent blank and the result was expressed as mg/dL.

2.8 Statistical analyses

Data were analyzed by SPSS® version 17 for Windows®. The results of continuous parameters are presented as Mean ± SD. Student’s t-test for independent samples was used to compare means of automobile workers and control groups. The Pearson coefficient of correlation was applied to examine the inter-relationship between Pb-B, renal function indices and various variables. The independent variables of smoking was entered as dummy variables and coded as 1 and 0. The participants who smoked were coded as 1 and the non-smokers were coded as 0. The regression analysis was used to examine the relationship between Pb-B and renal function indices and various variables. A $p$ value <0.05 was considered statistically significant.

3. Results

3.1 Baseline characteristics of the automobile workers and the controls

All of the total 45 automobile workers participated in this study were males. The mean age was 23.3 ± 8.7 years varied from 18 to 54 years. Average height of the workers was 158.1 ± 10.8 cm that varied from 141-173 cm and average weight was 50.7 ± 9.6 kg that varied from 33-69 kg. The mean body mass index (BMI) was 20.3 ± 4.09. Twenty one (46.6%) of the total automobile workers had smoking habit while 24 (53.4%) of them were non-smokers. Systolic and diastolic blood pressure (SBP and DBP) were measured at sitting conditions. The average systolic blood pressure of the workers was 125.33 ± 12.9 mmHg and diastolic blood pressure was 82.6 ± 9.6 mmHg. The control group had the mean age of 24.6 ± 5.5 years that varied from 21-39 years. The average height of the controls was 169.9 ± 5.2 cm that varied from 160-178 cm and average weight was 60.3 ± 7.3 kg that varied from 45-75 kg. The mean body mass index (BMI) was 21.3 ± 3.01. Of the total control subjects 14 (56%) were smokers and rest of them (46%) were non-smokers. It was found that the mean BMI was lower in the automobile workers compared to the controls but the difference was not significant (p=0.150). The average systolic blood pressure of the control subjects was 119.3
6.7 mmHg and diastolic blood pressure was 85.2 ± 8.1 mmHg. Table 1 shows all the baseline data of the study subjects.

### 3.2 Levels of serum lead in the automobile workers and the controls

It was found that the mean level of lead in serum of the automobile workers was 40.5 ± 22.75 µg/dL and the levels varied from 22.17-64.03 µg/dL with a median value 39.0 µg/dL. On the other hand, the mean serum lead level in the controls was 29.7 ± 10.1 µg/dL and the values varied from 5.10-45.37 µg/dL with a median value 33.75 µg/dL. Statistical analysis showed, serum lead level in the automobile workers was significantly higher (p=0.001) than the controls.

### 3.3 Levels of serum and urinary creatinine in the automobile workers and the controls

Of the total automobile workers, 35 (77.8%) had serum creatinine level within the normal range (0.7-1.4 mg/dL); and only 10 (22.2%) had lower level. The mean serum creatinine level in the automobile workers was 0.79 ± 0.13 mg/dL. All of the total 25 controls had serum creatinine levels within the normal range. The mean serum creatinine level in the controls was 0.97 ± 0.14 mg/dL. Statistical analysis showed serum creatinine level was significantly lower in the automobile workers (p < 0.001) compared to the control subjects. The mean calculated creatinine clearance (estimated Glomerular Filtration Rate, eGFR) was higher in the worker group (110.8 ± 30.8 mL/min) compared to control subjects (102.4 ± 20.5 mL/min) and these values did not show statistically significant variation (Table 1). The mean concentration of urinary creatinine was 83.8 ± 56.6 mg/dL, which was significantly lower (p = 0.003) than that of the controls 165.3 ± 118.7 mg/dL.

| Table 1: Comparison of the study variables between the automobile workers and control subjects |
|-----------------|-----------------|-----------------|-------------|
| Variables       | Workers Mean ± SD (Median), n = 45 | Controls Mean ± SD (Median), n = 25 | p - value |
| Age (yrs)       | 23.3 ± 8.7 (21.00) | 24.6 ± 5.5 (22.0) | 0.46 |
| BMI             | 20.3 ± 4.09 (19.04) | 21.6 ± 3.01 (21.3) | 0.15 |
| Systolic blood pressure, mmHg | 125.33 ± 12.9 (120) | 119.3 ± 6.7 (120) | 0.014 |
| Diastolic blood pressure, mmHg | 82.6 ± 9.6 (80) | 85.2 ± 8.1 (80) | 0.221 |
| Serum Lead (Pb-S), µg/dL | 40.5 ± 11.2 (39) | 29.7 ± 10.1 (33.75) | 0.001 |
| Serum Uric acid (mg/dL) | 6.6 ± 1.9 (6.1) | 6.9 ± 1.10 (6.8) | 0.332 |
| Serum Urea (mg/dL) | 31.0 ± 6.6 (30.84) | 37.1 ± 9.03 (37.01) | 0.005 |
| Blood Urea Nitrogen (mg/dL) | 14.5 ± 3.07 (14.3) | 17.3 ± 4.2 (17.3) | 0.005 |
| Serum creatinine (mg/dL) | 0.79 ± 0.13 (0.78) | 0.97 ± 0.14 (0.98) | <0.001 |
| Urinary creatinine (mg/dL) | 83.8 ± 56.6 (65.03) | 165.3 ± 118.7 (130.7) | 0.003 |
| GFR (mL/min) | 110.8 ± 30.8 (110.7) | 102.4 ± 20.5 (101.4) | 0.117 |
3.4 Levels of serum urea in the automobile workers and the controls

Forty four (97.7%) of the total workers had normal level (15-45 mg/dL) of serum urea; and 1 (2.3%) had an elevated level. The mean serum urea level in the automobile workers was 31.0 ± 6.6 mg/dL. Of the total 25 controls, 20 (80%) had normal level of serum urea; and 5 (20%) had elevated level. The mean serum urea level in the controls was 37.1 ± 9.07 mg/dL. Serum urea was significantly lower in the automobile workers (p=0.005) compared to the controls although both the mean values were within the normal range. Blood Urea Nitrogen (BUN) from the values of serum urea was calculated using following formula: Urea (mg/dL) = BUN (mg/dL) x 2.14. The values of BUN of the automobile workers and control subjects have been shown in Table 1.

3.5 Serum uric acid level in the automobile workers and the controls

Of the total automobile workers, 34 (79.1%) had serum uric acid level that was within the normal range (3.6 - 7.7 mg/dL); 9 (20.9%) had an elevated level. The mean serum uric acid level in the automobile workers was 6.6 ± 1.9 mg/dL. Of the total 25 controls, 18 (72%) had the normal uric acid level whereas 7 (28%) had an elevated level. The mean serum uric acid level in the controls was 6.9 ± 1.1 mg/dL. Statistical analysis revealed that serum uric acid was not significantly different (p=0.332) in the automobile workers compared to the control subjects.

4. Discussion

To our knowledge, this is the first study conducted to investigate the effects of serum lead on different biochemical parameters to evaluate renal functions among the adult Bangladeshi automobile workers. Numerous studies have demonstrated possible dysfunctions of different organs in occupationally exposed subjects using levels of lead present in whole blood (Perazalla, 1996; Ehrlich et al., 1998; Lin et al. 2002; Lin et al. 2001; Crawwell et al., 1984; Colleoni and fAmico, 1986) though measurement of lead in whole blood has weaknesses mainly in its non-linearity versus exposure effects (Al-Modhefer et al., 1991). The major weakness of the present study is the lack of data on the levels of lead in whole blood. However, serum lead level may better reflect kinetically more responsive and toxicologically labile fraction of circulatory lead that is more freely available to exchange with target tissues than do lead levels in whole blood (Smith et al., 2002; Bergdahl et al., 2006; Cake et al., 1996; Silbergeld et al., 1993). Free fraction of lead did not vary significantly with single day’s exposure (Bergdahl et al., 2006). Besides, anticoagulants such as EDTA, heparin can chelate lead (Al-Modhefer et al., 1991; Simons, 1993) which may result falsely high levels of lead in the plasma. Thus, serum was used to observe the chronic effects of free fraction of lead on different renal indices. Our data showed that the mean level of lead in the serum of control subjects was higher than reported by other studies (Bergdahl et al., 1999; Manton et al., 2001; Manton and Cook, 1984). This could be due to very high concentration of lead in the air (0.463 µg/m³) of Dhaka city (Khaliquzzaman et al., 1997; Kaiser et al., 2001). Control subjects enrolled in the present study were also residents of Dhaka city and used to live in the close proximity of major roads and intersections of the cities (data obtained from questionnaire) which could be one of the reasons behind the high levels of lead in their serum. Nevertheless, all the parameters except age, BMI and GFR reported in this study showed significant variation between the workers and controls (Table 1).

Renal ultrastructure in the proximal tubules, which re-absorbed two-thirds of the filtered load, has been found to be damaged due to lead as it is mainly excreted through the kidneys.
Modulation of renal function indices of the occupationally lead exposed Bangladeshi automobile workers (Loghman-Adham, 1997; Zenz, 1994). Weaver et al. (2005) have found association between lead exposure and lower urea, lower creatinine and higher creatinine clearance (associated with urinary creatinine) which represent lead induced hyperfiltration. In this study, we also found lower serum urea, creatinine, BUN and urinary creatinine but higher creatinine clearance in the lead exposed workers. About 22% automobile workers had a lower level of creatinine compared to the control subjects and the variation was statistically significant (p < 0.001). In this study, serum urea level was found normal in almost 98% of the automobile workers that supports the data of Ehrlich et al. (1998) who also found no effect of blood urea in lead-exposed battery workers. However, even within the normal range, the levels of serum urea in the workers were significantly lower compared to the controls. Urinary creatinine was found significantly lower (p = 0.003) in the automobile workers. Further, levels of serum creatinine showed a positive correlation (Spearman rho coefficient = 0.758; p < 0.05) with urinary creatinine which indicated that there is a relation between the production and excretion of creatinine in the workers. These data thus, suggested possible hyperfiltration of kidney in the automobile workers as reported by Weaver et al (2005).

![Figure 1: Scatter plots showing correlation of the levels of serum uric acid with creatinine clearance (A) and serum creatinine (B) of the automobile workers. Though serum uric acid is directly related with serum creatinine, a positive relation of uric acid with creatinine clearance indicated that uric acid is not associated with the impairment of kidney function. Lead has also been found to be associated with increased serum uric acid (Perazalla, 1996; Lin et al. 2002; Lin et al. 2001; Crawswell et al., 1984; Colleoni and fAmico, 1986; Campbell, 1978). In this study, concentrations of serum uric acid in the workers and controls were almost similar (Table 1). No relationship was observed between the levels of serum uric acid and Pb-S. This is an agreement with the finding of Alasia et al (2010) who also reported that Pb-B was not associated with serum uric acid. On the other hand, they reported that level of serum uric acid in lead exposed workers was positively correlated with serum creatinine and negatively related with estimated creatinine clearance. In the present study, level of serum uric acid of the automobile workers showed direct relation, though not significant,
with that of creatinine and glomerular filtration rate (Figure 1A and B, respectively). This further supports the possibility of lead induced hyperfiltration of the glomeruli of the workers.

A positive correlation between Pb-B and individual renal function index of BUN, serum creatinine, and urinary albumin was reported (Wang et al., 2002). Our data did not find any relationship between the levels of serum lead in automobile workers with any of the indices used to assess the renal function test. Also, regression analysis when performed considering the levels of serum lead as the dependent variable and renal function indices such as serum and urinary creatinine, serum uric acid, serum urea, BUN, and GFR as the independent variables, no relationship was observed. On the other hand, when regression analysis was performed by including other independent variables such as age, BMI, SBP, DBP, smoking along with renal function indices, significant positive association of serum lead with serum creatinine level and GFR, and negative relation with BMI were observed (Table 2).

Table 2: Linear Regression model of Pb-S, serum uric acid, renal function indices and other independent variables of the controls and workers

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Controls</th>
<th>Workers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficients (β)</td>
<td>p value</td>
</tr>
<tr>
<td>AGE</td>
<td>0.304</td>
<td>0.232</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.168</td>
</tr>
<tr>
<td>SCr</td>
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<td>0.813</td>
</tr>
<tr>
<td>BUN</td>
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<td>0.871</td>
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<tr>
<td>UCr</td>
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<tr>
<td>eGFR</td>
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</tr>
<tr>
<td>SBP</td>
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</tr>
<tr>
<td>DBP</td>
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</tr>
<tr>
<td>SU</td>
<td>0.433</td>
<td>0.108</td>
</tr>
<tr>
<td>SMOKING</td>
<td>0.387</td>
<td>0.215</td>
</tr>
<tr>
<td>R = 0.603</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.364</td>
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</tbody>
</table>

BMI: Basal Metabolic Index; Pb-S: Serum Lead; SCr: Serum Creatinine; BUN: Blood Urea Nitrogen; UCr: Urinary Creatinine; eGFR: estimated Glomerular Filtration Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SU: Serum uric acid.

5. Conclusion

In conclusion, present study showed that lead toxicity altered the normal levels of serum metabolites and in association with other factors such as BMI, blood pressure, habit of smoking that could contribute to the impairment of renal function in automobile workers. However, these results should be considered as preliminary data and more studies are warranted to elucidate the extent of influence of serum lead on the renal function indices.

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6. References


