Impaired renal and cellular functions in asymptomatic petroleum depot workers in Calabar metropolis, south-south Nigeria

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ABSTRACT
Location-determined diet/nutrition and environmental factors affect animal response to toxicants. This study aimed to ascertain the renal and cellular functions status in asymptomatic occupationally exposed and control male respondents in Calabar metropolis, a coastal area largely dependent on vegetables and sea foods, using standard protocols. The serum concentrations (mmol/L) in the exposed group for hydrogen carbonate, HCO₃⁻ (20.61±2.40) was relatively lower than in the control (21.95±1.64); whereas that for sodium ion, Na⁺ (141.31±3.52), potassium ion, K⁺ (4.54±3.77) and chloride ion, Cl⁻ (104.91±2.68), respectively were relatively higher than in the control (136.98±2.19, 3.64±0.44 and 96.27±5.09). The observations in the exposed group for sodium ion, Na⁺/K⁺ ratio (31.13±1.14), urinary creatinine clearance (µmol/L) (85.24±9.65) and urine creatinine concentration (µmol/L) (115.67±10.27), respectively were relatively lower than in the control (37.63±1.03, 91.88±7.97 and 119.96±10.97). The serum concentration for urea (3.19±0.84 mmol/L), creatinine (107.65±8.17 µmol/L), uric acid (0.31±0.05 mmol/L) and urea/creatinine ratio (0.03±0.12) respectively were higher than in the exposed group (4.27±1.28 mmol/L, 117.35±7.87 µmol/L, 0.44±0.07 mmol/L and 0.04±0.81). The observations apart from that for K⁺ were significant (p<0.05). Thus, the study suggests impaired renal and cellular functions in the petroleum depot workers, compares with that of similar but earlier studies and negates the possible location-determined diet/nutrition and environmental factors-associated modulatory influence on the depot workers responses to petroleum products fumes intoxication.

Key words: Renal function, cellular function, petroleum depot workers, uric acid, creatinine clearance

Introduction
Petroleum products vapour could persist in the environment (Schecter et al., 2006) and as pollutants could cause adverse effect on the environment and in humans (Marilena and Elias, 2008) with varying severity based on the chemical nature, concentration and persistence of the pollutant (Tietenberg, 2006). Thus, occupational exposure to petroleum products vapour will have significant adverse health implications. Depot workers in Calabar metropolis in Nigeria do not wear the personal protective kits which could worsen the adverse effects of petroleum products vapours exposure and the attendant health risks. Earlier studies abound (Awodele, et al., 2014; Al-Helaly and Ahmed, 2014; Festus, et al., 2013; Mahmood, et al., 2013), but were conducted in different locations.

However, it is a common knowledge that many factors, including location-determined diet/nutrition and environmental factors, may affect animals response to toxicants. Generally, Calabar, a metropolis in Cross River State, South-South, Nigeria, is a coastal area with the inhabitants largely inclined to eating vegetables and sea foods. Recent and similar studies (Egbuonu and Nkwazema, 2015; Egbuonu et al., 2015) suggested adverse influence in the liver, cardiovascular risks and impaired lipid metabolism.

These warranted this study aimed ascertaining the renal and cellular functions status in petroleum depot workers in Calabar metropolis, Nigeria, via set objectives, including the determination of urea, creatinine, chloride, sodium, hydrogen carbonate and potassium ions concentration in the serum and, for creatinine only, in the urine, and calculation of diagnostic ratios (Na⁺/K⁺ ratio and urea/creatinine ratio) from the value of corresponding serum results as obtained in this study. The kidney is an important organ for maintaining a constant extracellular environment by adjusting the excretion of water and electrolytes (Uboh et al., 2009). Thus, these bioindicators have been used to assess the physiological status, including the functional capacity of the kidneys, in animals (Egbuonu and Ezeanyika, 2013; Egbuonu...
and Osakwe, 2011; Egbuonu et al., 2010; Uboh et al., 2009; Egbuonu et al., 2009; Zannan et al., 2008; Nwankwo et al., 2006; Gidado et al., 2001).

**Materials and Methods**

**Chemicals and equipment**

The chemicals and equipment used were provided courtesy of the Chief Technologist, Chemical Pathology Laboratory, University of Calabar Teaching Hospital, Calabar Cross River State Nigeria. The chemicals were of analytical grade and products of reputable companies including Sigma-Aldrich, Germany and British drug House, London.

**Experimental design**

The participants, aged between 18 and 35, were asymptomatic male workers in 10 petroleum depots that do not provide the personal protective equipment and male University of Calabar students (for 2 to 4 years) in Calabar metropolis, Nigeria who are largely inclined to vegetable and sea food diets. The study adopted the sampling method of Lindell et al. (2001) and a standard questionnaire (Lippincot and Wilkins, 2000). The respondents were informed and their oral consent obtained before the administration of questionnaire and collection of blood and urine samples. Ethical clearance was sought, and approval obtained, from the Ethical Committee of Biochemistry Department, Michael Okpara University of Agriculture Umudike, Nigeria. Biochemical tests were conducted at the Chemical Pathology Laboratory of University of Calabar Teaching Hospital.

**Sample size determination and samples collection**

The determination of the sample size for this study, sixty four (64), and blood sample collection to obtain the serum was as described earlier (Egbuonu et al., 2015; Egbuonu and Nkwazema, 2015). Urine sample of each subject was collected into wide mouth, screw-capped, transparent containers.

**Biochemical tests**

**Flame photometric determination of serum sodium and potassium ions concentration**

The concentration of sodium and potassium ions in the serum was determined by flame photometric estimation of sodium and potassium, using compressed air. Dilute serum was sprayed as a fine mist of droplets (nebulised) into a non-luminous gas flame which emits the characteristic golden or lilac colour. Light of a wavelength corresponding to metal being measured (sodium or potassium) was selected by a light filter and allowed to fall on a photo-sensitive detector. The amount of light emitted is proportional to the concentration of metallic ions present.

In brief, 1,200 dilutions each of the sample and the standard for sodium and potassium were made in a universal container by diluting them with deionised water on the flame photometer and allowing them to warm for 15 minutes. The photometer reading was set at zero by using deionised water as blank. The equipment was calibrated with the diluted solutions of the standards to give 140 mmol/L for sodium and 5.0 mmol/L for potassium. The various test sample concentrations were then read on the digital read-out.

**Determination of serum chloride ion concentration**

The chloride ion concentration in the serum was determined by the method of Schales and Schales (1941). The principle was based on the colorimetric estimation of chloride which when titrated with mercuric nitrate solution, using diphenylcarbazone as indicator, gives a violet-blue complex that is measured colorimetrically and calculated using the relation:

\[
\text{Chloride ion concentration} = \frac{\text{Titration of test} \times \text{conc. of standard}}{\text{Titration of standard}}
\]

**Determination of serum hydrogen carbonate (bicarbonate) ion concentration**

The hydrogen carbonate ion in the serum was determined by the method of Van Slyke and Neill (1924). The principle was based on the colorimetric estimation of hydrogen carbonate in serum which when reacted with excess dilute \(H_2SO_4\) releases \(CO_2\) whereas the remaining \(H_2SO_4\) on titration with NaOH using neutral red as an indicator gives an orange coloured end point. The corresponding bicarbonate ion concentration was calculated using the relation:

\[
\text{Serum hydrogen carbonate ion concentration} = \frac{1}{\text{titre of test} \times 50 \text{ mmol/L}}
\]

**Determination of creatinine concentration in serum and urine**

The creatinine concentration in serum and urine was determined by the method of Barclay and Kenny (1947). This was based on the principle that picric acid (50 mmol/L) could remove protein from the serum sample while the creatinine in the resulting supernatant could react with creatinine buffer to form a yellow-orange colour. The absorbance of the yellow-orange could be read at 520 nm, and the creatinine concentration (in \(\mu\)mol/L) calculated from the relation:

\[
\text{Absorbance of test} \times \text{conc. of standard} = \text{Absorbance standard} \times \text{conc. of standard}
\]
The urinary creatinine clearance was calculated with Cockcroft formula as in Egbuonu and Ezanuika (2013), but for males and with assumption of uniform age.

**Serum urea concentration determination**

The serum urea concentration was determined by the method of Natelson (1951). This was based on the principle that urea could react with substances such as diacetyl in the presence of thiosemicarbazide and cadmium ions in acid conditions to form a rose-purple coloured solution. The absorbance of the resultant coloured solution could be measured at 540 nm, and the serum urea concentration (mmol/L) calculated from the relation:

\[
\text{Absorbance of test} \times \text{conc. of standard} = \text{Absorbance of standard}
\]

**Diagnostic ratios**

Diagnostic ratios, including Na⁺:K⁺ ratio and urea:creatinine ratio were calculated from the value of corresponding serum results as obtained in this study.

**Data analysis**

Data were analyzed by simple student t-test using statistical package and service solutions (SPSS) for windows version 7. Mean differences at p<0.05 were considered statistically significant. Results were presented as mean ± standard deviation (SD).

**Results**

The serum concentration (mmol/L) in the exposed group for hydrogen carbonate, HCO₃⁻ (20.61±2.40) was relatively lower than that in the control (21.95 ±1.64) by 6.11 % whereas that for sodium ion, Na⁺ (141.31±3.52), potassium ion, K⁺ (4.54±3.77) and chloride ion, Cl⁻ (104.91±2.68), respectively were relatively higher than that in the control (136.98±2.19, 3.64±0.44 and 96.27±5.09) by 3.16 %, 24.73 % and 8.94 % (Table 1). The observations apart from that for K⁺ were significant (p<0.05).

The observation in the exposed group for serum Na⁺:K⁺ ratio (31.13 ± 1.14) in Table 1, urinary creatinine clearance (µmol/L) (85.24 ± 9.65) and urinary creatinine concentration (µmol/L) (115.67 ± 10.27) (Table 2), respectively were relatively lower (p<0.05) than that in the control (37.63 ± 1.03, 91.88 ± 7.97 and 119.96 ± 10.97) by 17.27 %, 7.22 % and 3.58 %.

The serum concentration for urea (3.19 ± 0.84 mmol/L), creatinine (107.65 ± 8.17 µmol/L), uric acid (0.31 ± 0.05 mmol/L) and urea:creatinine ratio (0.03 ± 1.32) respectively in the control were relatively lower than that in the control (21.95 ±1.64) by 6.11 % whereas that for sodium ion, Na⁺ (141.31±3.52), potassium ion, K⁺ (4.54±3.77) and chloride ion, Cl⁻ (104.91±2.68), respectively were relatively higher than that in the control (136.98±2.19, 3.64±0.44 and 96.27±5.09) by 3.16 %, 24.73 % and 8.94 % (Table 1). The observations apart from that for K⁺ were significant (p<0.05).

**Table 1. Serum Na⁺, K⁺, Cl⁻ and HCO₃⁻ concentration and calculated Na⁺:K⁺ ratio in the control and exposed groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Control Group</th>
<th>Exposed Group</th>
<th>Difference relative to control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ concentration</td>
<td>mmol/L</td>
<td>136.98 ± 2.19</td>
<td>141.31 ± 3.52</td>
<td>+3.16</td>
</tr>
<tr>
<td>K⁺ concentration</td>
<td>mmol/L</td>
<td>3.64 ± 0.44</td>
<td>4.54 ± 3.77</td>
<td>+24.73</td>
</tr>
<tr>
<td>Cl⁻ concentration</td>
<td>mmol/L</td>
<td>96.27 ± 5.09</td>
<td>104.91 ± 2.68</td>
<td>+8.97</td>
</tr>
<tr>
<td>HCO₃⁻ concentration</td>
<td>mmol/L</td>
<td>21.95 ± 1.64</td>
<td>20.61 ± 2.40</td>
<td>−6.11</td>
</tr>
<tr>
<td>Na⁺:K⁺ ratio</td>
<td></td>
<td>37.63 ± 1.03</td>
<td>31.13 ± 1.14</td>
<td>−17.27</td>
</tr>
</tbody>
</table>

Result= Mean value ± SD for sample size, n = 64 *The difference was significant (p<0.05). ns The difference was not significant (p>0.05). + or – sign prefix denoted ‘increased by’ or ‘decreased by’, respectively.

**Table 2. Serum urea, creatinine and uric acid concentration, calculated serum urea:creatinine ratio and urinary creatinine clearance and urinary creatinine concentration in the control and exposed groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Control Group</th>
<th>Exposed Group</th>
<th>Difference relative to control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea concentration</td>
<td>mmol/L</td>
<td>3.19 ± 0.84</td>
<td>4.27 ± 1.28</td>
<td>+33.85</td>
</tr>
<tr>
<td>Creatinine concentration</td>
<td>µmol/L</td>
<td>107.65 ± 8.17</td>
<td>117.35 ± 7.87</td>
<td>+9.01</td>
</tr>
<tr>
<td>Uric acid concentration</td>
<td>mmol/L</td>
<td>0.31 ± 0.05</td>
<td>0.44 ± 0.07</td>
<td>+41.94</td>
</tr>
<tr>
<td>Urea:Creatinine ratio</td>
<td></td>
<td>0.03 ± 1.32</td>
<td>0.04 ± 0.81</td>
<td>+33.33</td>
</tr>
<tr>
<td>Urinary creatinine clearance</td>
<td>µmol/L</td>
<td>91.88 ± 7.97</td>
<td>85.24 ± 9.65</td>
<td>−7.22</td>
</tr>
<tr>
<td>Urinary creatinine concen</td>
<td>µmol/L</td>
<td>119.96 ± 10.97</td>
<td>115.67 ± 10.27</td>
<td>−3.58</td>
</tr>
</tbody>
</table>

Result= Mean value ± SD for sample size, n = 64 *The difference was significant (p<0.05). ns The difference was not significant (p>0.05). + or – sign prefix denoted ‘increased by’ or ‘decreased by’, respectively.

**Discussion**

Generally, location-determined diet and environmental factors affect animal response to toxicants, warranting this study in asymptomatic occupationally exposed and control male respondents in Calabar metropolis, a coastal area largely dependent on vegetables and sea foods. The serum concentration for urea, creatinine and uric acid in the exposed group were relatively higher than that in the control group, suggesting impaired excretion of these waste products that could either cause or worsen renal impairment (Kuehi et al., 2000; Edmunds et al., 2001; Sheth et al., 2006). These wastes along
with physiological electrolytes are usually eliminated through the urine, and their impaired elimination probably resulted in the higher serum electrolytes concentration in the exposed group observed in this study. In particular, high serum urea concentration suggested impaired kidney functions (Egbuonu et al., 2013; Siegel et al., 2004; Carriero et al., 2006) and the present result agrees with that of earlier studies (Nwanjo and Ojiako, 2007; Naza et al., 2013; Mahmood, et al., 2013; Awodele, et al., 2014) but, respectively in petrol/gasoline station workers and among petrol tanker drivers. Also, high serum uric acid concentration suggested renal impairment, in agreement with the result of earlier study (Uboh, et al., 2009) though in rats exposed to kerosene and gasoline vapours. It could, in addition, suggest enhanced tissue breakdown and release of intracellular nucleotides (Dykman and Simon, 1987), perhaps in apparent response to petroleum products vapours intoxication.

The significantly higher serum creatinine in the exposed depot workers as observed in this study agrees with that in gasoline filling station workers (Naza et al., 2013), petrol station workers (Festus, et al., 2013; Nwanjo and Ojiako, 2007), motor mechanics (Bartimaeus and Jacobs, 2003) and in rats exposed to kerosene and gasoline vapours (Uboh et al., 2009), suggesting impaired kidney functions due probably to enhanced production of creatinine. This may further suggest depletion in the intracellular arginine concentration and an inhibition of protein synthesis. Creatinine is a breakdown product of creatine whereas arginine is utilized in creatine biosynthesis (Markus and Rima, 2000) and concomitant decrease in the mRNA (involved in protein synthesis) content following high creatinine concentration has been reported (Guthmiller et al., 1994). The possible resultant depletion of arginine (a semi-essential amino acid that is very essential in nitric oxide synthesis) and inhibition of protein synthesis (vis a vis higher protein turnover suggested in this study) may be fundamental steps in the petroleum products vapour related toxicity, warranting follow up.

Electrolyte status provides an indication of the renal integrity and cellular functions in animals (Uboh et al., 2009). The decrease in HCO\(_3\) observed in this study contrasted with the increase reported earlier, but in petrol station attendants in Owerri, Nigeria (Festus, et al., 2013). Generally, hydrogen carbonate, HCO\(_3\) maintains the acid-base balance, hence the observed lower concentration in the serum of the exposed group may be indicating compromised physiological buffering integrity in the exposed group, perhaps in response to apparent adverse effect following higher concentration and longer exposure of the depot workers to petroleum products. The other studied serum electrolytes were higher in the exposed group than in the control. The observation was, aside that of K\(^+\), significant (p<0.05), seemingly indicating decreased excretion of these electrolytes (due perhaps to kidney and cellular dysfunctions) that may result in pathological conditions. For instance, high blood Na\(^+\) and Cl\(^-\) concentration was associated with increased blood pressure (Overlack et al., 1993; Boegehold and Kotchen, 1991). In contrast to the result of the present study, Festus et al. (2013) and Uboh et al. (2009) reported a decrease in the Na\(^+\) and Cl\(^-\) concentration but in petrol station attendants and in rats exposed to kerosene and gasoline vapours, respectively. The higher Na\(^+\) and Cl\(^-\) concentration in the exposed group of this study could be due to the higher concentration of, and longer exposure to, the petroleum products (Okoro et al., 2006) that could adversely alter the electrolyte balance of the unprotected petroleum depot workers.

The observation in the exposed group for the calculated serum Na\(^+\)/K\(^+\) ratio was relatively lower (p<0.05) than that in the control by 17.27 %, suggesting compromised Na:\(\text{K}^+\) ATPase activity that may result to the general body weakness complained by the exposed subjects. The higher Na\(^+\) and K\(^+\) observed in the exposed group could up-regulate the activity of ATP-dependent Na:\(\text{K}^+\) ATPase pump that could, over time, deplete the available ATP consequently resulting in compromised Na:\(\text{K}^+\) ATPase activity and general body debility. Urea:creatinine ratio was higher (p<0.05) in the exposed group by 33.33 %, suggesting higher protein turnover (Egbuonu et al., 2010) and decreased muscle mass (Feinfeld et al., 2002) due perhaps to abnormal protein catabolism following compromised kidney integrity. The relatively lower (p<0.05) urinary creatinine concentration and creatinine clearance in the exposed than in the control suggested decreased muscle mass (Egbuonu and Ezeyanika, 2013) which may increase mortality risk in the exposed subjects. Low muscle mass independently predisposed, though older, men to high mortality (Wannamethee et al., 2007).

**Conclusion**

The study suggests impaired renal and cellular functions in the petroleum depot workers, compares with that of similar but earlier studies and thus, negates possible location-determined diet/nutrition and environmental factors-associated modulatory influence on the depot workers response to petroleum products fumes intoxication. The study underscores the need for the workers to wear personal protective equipment and to regularly assess their health status.

**References**


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Low dose oral administration of monosodium glutamate in male albino rats may be nephroprotective. Bio-Research 7(1): 470-473.


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