Effects Of Prolonged Exposure To Gas Flare On Renal Functions Status Of Adult Humans In Finima, Bonny Island

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Abstract: Residents of Finima community, Bonny Island, in Nigeria’s Niger Delta Region, for so many years now, have been exposed to the hazardous environmental conditions occasioned by the operations of oil and gas industry. And typical of this, is gas flaring. The flared gases as by-products of incomplete combustion are known to overwhelm the environment with numerous dangerous pollutants. Ultimately, the contamination of man’s food and water sources by these pollutants may trigger several severe health effects. The kidneys and the liver, by their natures are primarily endangered by most external pollutants. Considering the reports and realities on ground of the impact of long-term exposure to flared gases on both humans and the environment, it became imperative to embark on an in depth study based on established biomarkers to underscore the influence of exposure to flared gases on the renal functions of adults resident in Finima, Bonny Island. The individuals recruited as test subjects were those who have resided in this gas flare impacted environment for up to fifteen years and above. The values of this set of subjects were compared to control subjects drawn from non-oil and gas production environments. The subjects (400) were matched for age and sex, and sorted into exposed group (200) and control group (200). Blood samples were obtained from the subjects and analysed for serum levels of sodium, potassium, chloride, urea, creatinine and cystatin-C. The results revealed that individuals exposed to gas flare impacted environment had significantly (p<0.05) higher serum levels of cystatin-C, creatinine, urea, potassium, sodium and chloride with respect to the values of control subjects. The above results suggest that a long time exposure to low level of gas flare may lead to significant elevations of dysfunctional biomarkers of the kidneys and thus, may be predisposed to having renal derangements.

Keywords: Gas flares, Cystatin-C, renal biomarkers, Finima, Bonny Island, Niger Delta

1. INTRODUCTION

Finima is a community in Bonny kingdom and is located along Latitude 4.417° North and Longitude 7.150° East [26, 41] with its political boundary situated within Bonny Local Government Area of Rivers State. The community and its environs have been berated by most environmental scientists in Nigeria; United Kingdom; the United States of America, amongst others, as one of the most polluted areas of the world. A big contributor to this poor environmental situation has been identified to be the constant release of associated gases during the process of oil production. This, of course, has tremendous impact on the ecology and humans alike in the environment, thus, making the region unhealthy to inhabit. Gas flaring is a term used to describe the process of release of associated gases from the operations of refineries, wells, or hydro-carbon plants, this could be to dispose these gases or as a safety measure to lessen pressure [34, 43]. Flaring is undertaken because of the expensive nature to segregate commercially variable associated gases from the oil, due to unavailability of the requisite technology for conserving and utilising flared gas [11, 27]. Records have it that amongst the numerous oil flow stations situated in the Niger Delta area of Nigeria over 400 of them are located onshore; 251 on offshore with 5,284 barrels being the total drilled oil flow stations and oil and gas pipelines run across 7,000 km [5, 16]. Furthermore, the flaring sites in the country are over 123 with about 20 of which are located in Finima, Bonny Island, thereby making her a major flaring locality in Nigeria and amongst the chief emitters of greenhouse gases in Africa [5]. The flaring of gas in Nigeria is considered a national predicament and this is because, the price of sustained gas flaring is unquantifiable. For example, associated gas that is a potential source of energy is flared and wasted. Thus, a huge source of revenue is going up in flames. In 1998, Ashton-Jones [5] reported that about half a million Naira is lost to gas flaring daily in Nigeria. Statistics indicate that Nigeria accounts for about 28% of the total amount of gas flared globally [32]. Due to incomplete combustion during the process of gas flaring, so many poisonous gases are released [10], and this include polycyclic aromatic hydrocarbons such as benzene, napthalene, styrene, acetylene, fluoranthene, xylene, pyrene and ethylene [10]. The act of flaring produces different harmful emissions such as particulate matter like soot or black carbon [30] that has adverse impact on the quality of air [17, 40]. These unstable organic compounds that are taken into the systemic circulation through the air ways, or the food chain, [12] have a variety of potential influence on the health, man and his animals; particularly, when they are bioactivated through the activity of various cytochrome p450 enzymes systems located in various tissues [8]. The volatile organic compounds released during oil and gas exploration have been established to be hazardous to
animals. The production and release of high volumes of these volatile organic compounds usually lead to substantial emission into the environment. Due to the abundance of heavy metals like zinc, barium, iron, cobalt, manganese, selenium, cadmium, nickel, aluminium, vanadium and lead in crude oil and flared gas [15], the frequent gas flaring and spills of crude oil on land and water may be a major cause of heavy metal contamination in terrestrial habitats of oil and gas producing community of Niger Delta of Nigeria [15]. One of the primary target organs of toxicity from chronic exposure to products of gas flares and/or their biotransformed forms is the kidney. It is particularly sensitive to such heavy metals as cadmium, mercury, lead, and halogenated hydrocarbons [3, 11]. The effect on metabolism of the volatile hydrocarbons, including the flared gases is receiving more attention due to their toxic potentials in the internal organs of the human body. Over time, the serum levels of creatinine and blood urea nitrogen and urinalysis, formed the basis of evaluation of the efficiency of kidney functions [25]. However, accumulating evidences have demonstrated that these biomarkers are not optimal to detect renal disease in early stages [6, 7, 25, and 45]. However, cystatin-C is one biomarker that is not affected by either endogenous or exogenous factors, thus serving a more reliable role in the evaluation of the functional status of the kidney. The present study therefore aims to evaluate the serum levels of cystatin-C, urea and creatinine and electrolytes amongst some residents of Finima community (as a typical gas flaring and oil spill impacted area) in order to ascertain their health status.

2.0 MATERIALS AND METHODS

2.1 Ethical Approval
Ethical approval was obtained from the Ethics and Scientific Committee of University of Port Harcourt as well as Finima Health Centre, Bonny Island and Meridian Hospitals Port Harcourt before embarking on the study.

2.2 Consent from Human Subjects
Dully signed consent forms were obtained from all the test and control human subjects in the study.

2.3 Exclusion Criteria
The study population did not include human subjects who were aged below 18 and above 60 years, had a positive history of more than one health complications, were on special medication for any disease, did not give consent, had still born babies, are smokers, had history of alcohol abuse, work outside Finima community for the test cases and must not have been to Finima Bonny Island or any other community anywhere in the world where oil and gas exploration is taking place. (This is only applicable to samples collected at Meridian Hospitals Port Harcourt which shall be acting as the control).

2.4 Inclusion Criteria
Below are the inclusion criteria for the human subjects in this study: Human subjects within the age group of 18-60 years, apparently healthy subjects with no history of recent blood transfusion, apparently healthy subjects and subjects with no clear diagnosis of other known diseases, apparently healthy subjects who have been residing in the study area (Finima Bonny Island) for over 15 years (for the test group) and have been to Finima Bonny Island or any other community anywhere in the world where oil and gas exploration is taking place (for the control group, whose samples were collected at Meridian Hospitals Port Harcourt).

2.5 Study Population
This is a cross sectional case control study involving two separate populations (Finima community and Port Harcourt) located in Niger Delta region.

Study Population:
Sample size was determined using Taro Yamane minimum sample size determination formula:

\[ n = \frac{N}{1 + N(e)^2} \]

Where N=Estimated size of the population; n=sample size; e=level of precision or error of sampling at ±5% [46]. A total number of 400 human subjects were recruited for this study, which were within the ages of 18 and 60 years comprising 200 test and 200 control subjects. Blood samples were collected from the participants at Finima Health Centre located in Bonny Kingdom (used as the test group), and Meridian Hospitals located at Igbokwu street Port Harcourt (served as the control group). The samples were analysed at the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Choba, Rivers State, Nigeria.

2.6 Specimen Collection and Processing
Blood specimen was collected from a peripheral vein via antecubital venipuncture from the respective human subjects. This was dispensed into an SST vacuum tube and then centrifuged for 10 minutes at 1000 rpm. The serum was separated from the cells and transferred into plain sample bottle and then frozen at -20 °C in deep freezer until the time for analysis within thirty days of collection.

2.7 Chemicals and Equipment
The kits used for the measurement of serum urea and creatinine was procured from Mindray Bio-Medical Electronics Co., LTD, China; that for cystatin-C was obtained from Elabscience Biotechnology LTD, Wuhan, Hubel Province, China; while electrolyte reagents were obtained from Genus, a subsidiary of Diamond Diagnostics, USA. All the chemicals and kits were of analytical grade. Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Mindray BS-800M Clinical Chemistry Analyzer (China) was used for the measurement of urea and creatinine. Genus GE-200 Electrolyte Analyzer, from Diamond Diagnostics (USA) was used for the determination of sodium, potassium, and chloride ions while Stat Fax®4200 Microplate Elisa Reader from Awareness Technology, Palm City, Florida (USA) was used to measure cystatin-C concentration.

2.8 Analytical methods
Creatinine was determined by the Jaffé-Slot method [23], while urea was determined by urease-glutamate dehydrogenase, UV method [42]; both kits were from Mindray Bio-medical Electronics Co., LTD (China).
2.9 Statistical analysis of data
The raw data collated by the present study was analysed using Statistical Package for Social Sciences (SPSS) version 17. The differences across the parameters evaluated between the various groups were determined using the Student’s t-test. ANOVA was used to assess differences within the groups. A non-parametric method was adopted to determine the reference intervals (RIs) as specified by IFCC (International Federation of Clinical Chemistry) guidelines. The limits of the conventional 95% RI have rank numbers equal to 0.025 × (n + 1) and 0.975 × (n + 1). The values corresponding to these rank numbers are the RI. Statistically significant values were determined at p <0.05 or 95% confidence level.

Upper reference limit = mean + $t_{0.975,(n-1)} \frac{n + 1}{n}$ SD

Lower reference limit = mean - $t_{0.975,(n-1)} \frac{n + 1}{n}$ SD

Where n =100; $t_{0.975, (100-1)}$; $t_{0.975, (99)}$ = 1.9797 from t-test table.

3.0 RESULTS
Table 1.1 shows the serum electrolytes levels of the exposed and non-exposed males and females in the study areas. The results showed a statistically significant (p<0.05) increase in sodium level in the exposed subjects compared to the non-exposed subjects. The serum potassium levels of the males and females in the exposed group, were significantly (p<0.05) higher than those of the corresponding males from the exposed group. The serum chloride levels of the exposed and non-exposed males and females in the study areas are equally shown in Table 1. There was a significant (p<0.05) increase in the exposed subjects compared to the non-exposed. Compared to the various control or non-exposed group, the serum potassium levels of the males and females in the exposed group, were significantly (p<0.05) higher. The serum potassium levels of the females in the exposed and non-exposed groups were significantly (p<0.05) lower than those of the corresponding males from the exposed and non-exposed groups. The serum chloride levels of the exposed and non-exposed males and females in the study areas are shown in Table 1.1. The result revealed significant (p<0.05) increase in the exposed subjects compared to the non-exposed. Compared to the various control or non-exposed group, the serum potassium levels of the males and females in the exposed group, were significantly (p<0.05) higher. The serum potassium levels of the females in the exposed and non-exposed groups were significantly (p<0.05) lower than those of the corresponding males from the exposed and non-exposed groups.

Table 1: Comparison of serum electrolytes levels in exposed and non-exposed males and females.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (mmol/L)</th>
<th>Males</th>
<th>Females</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexposed</td>
<td>Exposed</td>
<td>Unexposed</td>
<td>Exposed</td>
</tr>
<tr>
<td>Sodium</td>
<td>141.21±6.79</td>
<td>145.14±7.10</td>
<td>140.72±6.68</td>
<td>144.98±6.49</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.23±0.66</td>
<td>4.68±0.67*</td>
<td>4.19±0.70*</td>
<td>4.44±0.67*</td>
</tr>
<tr>
<td>Chloride</td>
<td>102.31±3.53</td>
<td>106.82±4.34</td>
<td>102.43±3.27</td>
<td>105.26±4.20</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviations, n = 100 (except for combined with n=200).

*aP<0.05 compared to corresponding control (unexposed group)

*bP<0.05 compared to corresponding male value

Table 2 shows the serum urea levels of the exposed and non-exposed males and females in the study areas. It showed a statistically significant (p<0.05) increase in the exposed subjects, compared to the non-exposed subjects. The serum urea levels of the males and females in the exposed group, were significantly (p<0.05) higher than those of the corresponding control or non-exposed group. The serum urea levels of the non-exposed females was significantly (p<0.05) lower than that of the non-exposed males but there is no significant difference in the exposed males and females. Table 3 shows the serum creatinine levels of the exposed and non-exposed males and females in the study areas. The results showed a statistically significant (p<0.05) increase in serum creatinine level in the exposed subjects compared to the non-exposed. The serum creatinine levels of the males and females in the exposed group, were significantly (p<0.05) higher than those of the corresponding control or non-exposed group. The serum creatinine level of the non-exposed females was significantly (p<0.05) lower than that of the non-exposed males but there is statistically significant increase in the exposed males compared to the exposed females in the study. The serum cystatin-C levels of the exposed and non-exposed males and females in the study areas are shown in Table 4. It showed a statistically significant (p<0.05) increase in cystatin-C in the exposed subjects compared to the non-exposed control subjects.
Compared to the corresponding control or non-exposed groups, the serum cystatin-C levels of the males and females in the exposed group, were significantly (p<0.05) higher. The serum cystatin-C level of the females in the non-exposed groups was significantly (p<0.05) lower than that of the corresponding males from the non-exposed group while there was a significant increase in the exposed females compared to the exposed males. Table 5 shows the comparison of reference range for males and females obtained from this study, with results of males and females obtained from pre-existing reference ranges. The male subjects showed a reduced lower value of sodium, potassium, urea, creatinine, and chloride levels but higher level of cystatin-C in the study reference but none in the upper ranges except chloride, urea, creatinine and cystatin-C that showed a higher value in the study reference. The table equally showed a reduced level of the lower reference of sodium, potassium, chloride, urea, creatinine, and cystatin-C in the study reference compared to the pre-existing female reference but a higher value in the upper reference of chloride and cystatin-C in the study reference compared to the pre-existing reference range.

Table 2: Comparison of serum urea levels in exposed and non-exposed males and females.

<table>
<thead>
<tr>
<th>Concentration (mmol/L)</th>
<th>Unexposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>4.82±2.20</td>
<td>6.47±2.03*</td>
</tr>
<tr>
<td>Females</td>
<td>4.19±1.97b</td>
<td>6.23±2.49a</td>
</tr>
<tr>
<td>Combined</td>
<td>4.50±2.11</td>
<td>6.35±2.27a</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviations, n = 100 (except for combined with n=200).
*P<0.05 compared to corresponding control (unexposed group).
bP<0.05 compared to corresponding male value

Table 3: Comparison of serum creatinine levels in exposed and non-exposed males and females.

<table>
<thead>
<tr>
<th>Concentration (µmol/L)</th>
<th>Unexposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>69.33±15.20</td>
<td>104.89±8.39</td>
</tr>
<tr>
<td>Females</td>
<td>57.38±15.91</td>
<td>103.33±8.28</td>
</tr>
<tr>
<td>Combined</td>
<td>63.40±16.63</td>
<td>104.11±8.35</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviations, n = 100 (except for combined with n=200).
*P<0.05 compared to corresponding control (unexposed group).
*P<0.05 compared to corresponding male value

Table 4: Comparison of serum cystatin-C levels in exposed and non-exposed males and females.

<table>
<thead>
<tr>
<th>Concentration (µg/L)</th>
<th>Unexposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>172.02±46.85</td>
<td>415.45±50.48</td>
</tr>
<tr>
<td>Females</td>
<td>136.21±36.27</td>
<td>420.76±60.38</td>
</tr>
<tr>
<td>Combined</td>
<td>154.10±45.48</td>
<td>418.11±55.57</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviations, n = 100 (except for combined with n=200).
*P<0.05 compared to corresponding control (unexposed group).
*P<0.05 compared to corresponding male value

4.0 DISCUSSION

From the outcome of this study, the analysis of serum sodium level in the exposed male and females showed a significant increase in the exposed subjects when compared with the unexposed subjects. Increase in sodium level could be as a result of dehydration, severe vomiting, prolonged diarrhoea, profuse sweating, fever, polyuria, hyperaldosteroidism and inadequate water intake. This finding is in agreement with the report of Oseji [37] which stated that gas flaring results in increased ambient temperature. Recall that Kadafa [18-19] and Aaron [1] had reported that about 45.8 billion kW of heat are discharged into the atmosphere of the Niger-Delta, due to the flaring of about 1.8 billion cubic feet of gas every day. Increased ambient temperature can cause persistent insensitive loss of body fluid, thus leading to chronic dehydration among resident of gas flared environment which can lead to reduced blood volume, rise in blood viscosity and blood pressure. Nwankwo and Ogbeibu [31] reported that dehydration is further worsened by the poor water quality in the region as there is dearth of potable water to checkmate the effect of fluid loss from the body. This finding is equally in tandem with Egwurugwu et al., (2013a) [11] that reported that chronic and persistent dehydration emanating from heat generated as a result of gas flaring can lead to reduced glomerular filtration rate and a rise in serum sodium ion level. The level of serum creatinine showed a statistically significant increase in exposed males and females when compared to the control. High creatinine could be as a result of renal failure, prostatic diseases, cancer of the bladder, drugs such as salicylate, acetate from patients with diabetic ketoacidosis. Creatinine is freely filtered, secreted just like urea, are not affected by protein diet but changes significantly according to the muscle mass of the individual. This finding agrees with Egwurugwu et al., (2013a) [11] that noted an increase in creatinine level among individuals exposed to the toxic effect of gas flares in Imo state as a result of chronic and persistent dehydration emanating from heat leading to reduced renal perfusion and reduced glomerular filtration rate. The increase is higher in male than in female which could equally be explained in terms of high CPY2E1 oxidation in males than in females and the fact that men in Finima community tend to be close to the field where gas flaring is occurring than their female counterpart. Loghman-Adham [24] and Orisakwe et al., (2004) [36] had reported that kidneys can be greatly damaged before losing sufficient function to modify the normal clinical indication of renal disease, because more than 50% of renal capacity may be lost before serum creatinine becomes abnormal and disease is detected clinically. Thus, creatinine which is known to be influenced by the amount secreted and/or reabsorbed and muscle mass, cannot give an accurate measure of glomerular filtration rate. Analysis of serum cystatin-C level showed a statistically significant increase in the test subjects compared to the controls. There has been no reported case linking cystatin-C with gas flaring effect but Anderson et al. (2009) [4] had reported that cystatin-C increased in the serum of workers exposed to industrial chemicals, while Knight et al. (2004) [21] had noted that the concentration of cystatin-C is not affected by age, gender, race, protein intake and muscle mass unlike urea and creatinine. Since cystatin-C is formed at a constant rate and is freely filtered by the kidney but not secreted or reabsorbed, and cystatin-C inversely correlates with...
glomerular filtration rate, meaning that increase in cystatin-C leads to reduced glomerular filtration rate. The increase in cystatin-C could be attributed to the toxic effect of gas flared constituents. These constituents serve as secondary metabolites which are generated during oxidation of benzene and benzene-like compounds by cytochrome P450 enzymes that are well known electrophiles which readily react with peptides, proteins, DNA and forms protein adducts. The formation of these metabolites have been identified in parallel with poly aromatic hydrocarbon (PAH) exposure, and the formation of these adducts might provide a more accurate assessment of PAH exposure and potential nephrotoxicity. The analysis of serum cystatin-C showed a gender difference with females having a higher level than the male which negates an earlier position by Adamu (2015)[2] and Knight et al. (2004) [21] which reported that cystatin-C levels are not statistically affected by gender but agrees with Okonkw et al. (2015) [33] that noted an increase in serum cystatin-C in females compared to the males.

5.0 Conclusions
This study has shown that the evaluation serum level of cystatin-C can be a more reliable biomarker for renal toxicity. Consequently, cystatin-C should be incorporated alongside serum electrolyte, urea and creatinine to assess the status of renal functions. Again, this study has revealed that adults in Finima community that are exposed to gas flare for a prolonged period may be more predisposed to having renal functions derangement.

6.0 Acknowledgment
The authors are grateful to Dr (Mrs) Ogunka-Nnoka, HOD, Biochemistry, University of Port Harcourt, Rivers State Nigeria for encouraging us to embark on this area of research, and Mr Benjamin Aleme of the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Rivers State Nigeria for assisting us in the analysis of the specimens.

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