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Heavy Metal Levels and Protein Oxidation Toxicity in People Living in a Gas Flared Environment

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Abstract

This study was carried out on blood samples collected from 200 residents each from Imiringi (Gas Flaring Community) and Odi (Non-Gas Flaring Community) all in Bayelsa State, Nigeria to determine the blood levels of cadmium, chromium, lead, mercury and selenium as well as protein radicals.. Blood samples were also analyzed for total WBC, Hb, platelets, prothrombin time and serum concentrations of AST, ALT, albumin, ALP, bilirubin, gamma GT, total protein, sodium, potassium, chloride, bicarbonate, urea and creatinine. The highest blood levels of protein radicals, mercury, lead, selenium, cadmium and chromium were observed in people living in in Imiringi community and the observed values were all statistically significantly different at $p<0.05$. Residents in Imiringi also had the highest mean values of all the measured liver and kidney functions parameters with the exception of albumin, total protein, sodium, chloride and bicarbonate and these values were all statistically different at $p<0.05$. There was statistically significant difference in the levels of Hb, WBC and Platelet while prothrombin time was prolonged in the residents of Imiringi community. The findings of this study suggest that the probability of occurrence of diseases associated with metal toxicity and protein oxidation processes could be higher among residents in the gas flaring community.

Keywords: Heavy Metal, Gas Flaring, Protein Oxidation, Reactive Oxygen Species, Oxidative Stress

I. INTRODUCTION

Nigeria being the largest crude oil producer in Africa and the eighth in the Organization of Petroleum Exporting Countries (OPEC) ranking, [1] has witnessed a large amount of pollution problems associated with oil production activities since oil was discovered in commercial quantity in 1956. Majority of these pollution problems are due to spillage of petroleum products into the surrounding environments, disposal of associated wastes and flaring of natural gas.

During the process of producing oil, billions of cubic meters of natural gas is flared or vented annually at different oil production sites world-wide. Gas flaring sites in Nigeria are located mainly in the Niger Delta region where the bulk of petroleum mining and refining activities take place. These sites are often located near public facilities, homes, schools, farms, and within host communities.

Gas flaring is a significant source through which greenhouse gases (water vapor (H2O), carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O), and ozone (O3)) as well as sulfur dioxide (SO2) are emitted into the atmosphere. These substances mix and react with water, oxygen and other chemical pollutants to form acid rain which is very common in the Niger Delta region [2]. Gas flaring also generates noise, heat and cause large areas of land to be uninhabitable. Volatile and non-volatile heavy metals such as Lead (Pb), Mercury (Hg), Cadmium (Cd) and Arsenic (As) have been found to be released into the surrounding environment as a result of flaring of natural gas [3] Heavy metals contamination of the soil and rain-water samples obtained from gas flared

environments are also evident. The consequent effects of gas flaring on these locations and its inhabitants are numerous. Notable effects are health hazards (such as skin problems, cancer, reproductive health problems and respiratory disorders), poor soil fertility, climate change (bringing about flooding) and economic loss. [2]

Although there is considerable public concern about the environmental impacts of oil pollution in the Niger Delta region of Nigeria, actual evidence on the pathological and physiological effects on the health of local inhabitants is minimally known. We therefore in this study, sought to associate the perspective measures of exposure to oil pollution with health outcomes of residents in affected locations.

Heavy metals are quintessential to maintain various basic biochemical and physiological functions in living organisms when in very low concentrations. However, they become noxious when they exceed certain threshold concentrations.These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity [4]. Reactive oxygen species(ROS) are routinely produced as a by-product of aerobic metabolism and oxidative phosphorylation. In addition, ROS production and accumulation are usually increased during disease pathogenesis (i.e., in particular age-related diseases) [5]. Low concentrations or transient exposure to ROS induce cell proliferation and regulate the activation of several signalling pathways [6]. However, un-neutralized ROS cause oxidative damage to lipids, proteins, and nucleic acids, thus leading to aberrant molecular activities [7]. Protein oxidation is particularly detrimental as the resulting damages and/or induced conformational changes to protein structures can render oxidized proteins inactive and lead to cellular functional abnormalities. Carbonylated proteins are generally less active, less thermo-stable and expose hydrophobic amino acids at their surfaces. Oxidative modifications that give rise to carbonyl groups generally
cause loss of catalytic activities [8]

Various types of protein oxidative modifications are induced directly by ROS or indirectly by reactions with secondary products of oxidative stress [9]. The exponential rate of accumulation of carbonylated proteins during life span both at the cellular and organismal levels and their particular increase in organs affected by age related diseases, imply that this "Oxi-proteome" (i.e., the restricted set of proteins targeted by oxidation) may be a potential molecular substratum for many of the cellular dysfunctions described [10]. Protein carbonylation includes aldehydes and ketones formed via different mechanisms:
(i) direct oxidation of the polypeptide backbone leading to truncated peptides; (ii) side chains oxidation of lysine, arginine, proline, and threonine; (iii) reaction of histidine, cysteine, and lysine amino acid residues with aldehydes usually derived from lipid peroxidation; and (iv) glycation (nonenzymatic glycosylation) of lysine residues forming Amadori and Heyns rearrangements products [10] Elevated levels of proteins modified by lipid oxidation products (4-hydroxy-2-nonenal: HNE, malondialdehyde) are associated with neurodegenerative diseases, iron-induced renal carcinogenesis, cardiovascular disease. Elevated levels of protein glycation/glycoxidation end products (advanced glycation end products (AGEs) are associated with diabetes mellitus, neurodegenerative diseases, atherosclerosis, and Down's syndrome [11]. Significant advances in recent years have been made towards the identification of proteins targeted by these modifications, although their possible causative role in the pathogenesis of these diseases has not yet been determined.

From the foregoing, it becomes expedient to assess the levels of heavy metals and protein oxidation marker (protein radicals) in people living in gas flared environments that are exposed to crude oil pollution, drink from the polluted water, eat plants harvested from the surrounding soil and equally consume aquatic animals found in these environments. Considering the cumulative health effects that may result from these exposures, this study tried to investigate the impact of gas flaring on human health to improve abating efforts and also to prevent adverse health effects on individuals living in affected locations.

II. METHODOLOGY

Study Area

This study was carried out in two communities in Bayelsa state, Nigeria.

(a) Imringi Community (Gas Flaring Community). This community is located within Kolo Creek in Ogbia Local Government Area of Bayelsa State, Nigeria.

(ii) Odi (Non-Oil Producing Community).The community is located in Kolokuma/Opokuma Local Government Area of Bayelsa state, Nigeria.

Both communities are located in Bayelsa State which lies within, latitude 040 151 North, 050 231 South and longitude 050 221 West and 060 451 East [12]. Imiringi is the test community while Odi community was used as control.

Study Population

A total number of 400 subjects were recruited for this study. This comprised:

200 residents of Imiringi community (Gas flaring area) and 200 residents from Odi (Non-gas flaring community).

The subjects from Imiringi community comprised of 84 males aged between 2 and 80 years and 116 females aged between 2 and 78 years. In Odi community, 106 were males aged between 2 and 80 years while 94 were females aged between 2 and 79 years old.

Advocacy and Mobilization

The Bayelsa State Ministry of Health granted the ethics clearance for this study. The traditional rulers and leaders of the community development committees of each community gave consent to the study after due consultations and also informed consent was obtained from the subjects recruited for the study.

Selection Criteria

Questionnaire was used to obtain the required information needed for including or excluding participants.

Inclusion Criteria

Subjects two years and above that consented to the study were included. The sample population was classified according to sex and age groups.

Exclusion Criteria

Subjects with known illnesses such as cancer, diabetes mellitus and Parkinson's disease were excluded. Since cigarette smoke is an exogenous source of oxidative stress, smokers were equally excluded from this study [13]. while levels of oxidative stress biomarkers are known to be raised in the above mentioned disease conditions [14].

Sample Collection

Blood samples were collected by venipuncture using sterile disposable syringes. Samples for measurement of protein radicals and biochemical parameters were collected in plain serum separating tubes. These were allowed to stand for 10-20 minutes after which they were centrifuged at 3,000 rpm for 20 minutes and the serum separated using a Pasteur's pipette. Samples for measurement of hematological parameters (prothrombin time not inclusive) and heavy metals were collected in K_3EDTA anti coagulated bottles and mixed thoroughly by gentle repeated turning. Samples for prothrombin time were dispensed into containers containing 3.2% tri sodium citrate at a ratio of 9 parts of blood to 1 part of 3.2% tri sodium citrate. The samples were centrifuged for 15 minutes at 3000 rpm to obtain platelet poor plasma. The supernatant plasma was subsequently transferred into plain eppendorf tubes.

Laboratory Procedures

All the reagents used for this study were commercially purchased from Afro Famous Nigeria Limited, Abakpa Nike, Enugu, Enugu State, Nigeria and all manufacturers' SOPs were followed strictly.

Protein Radicals [14]. The Elabscience protein radicals ELISA technique test kit was used for the quantitative measurement of protein radicals in the test subjects.

Measurement of Cd, Cr,Hg, Pb and Se.

Measurement of these metals was done with 240 FS AA Agilent Technologies flame atomic absorption spectrometer with deuterium lamp background correction.

Alanine Aminotransferase [15]
RANDOX ALT kit was used.

(D) Aspartate Aminotransferase (AST) [16]
RANDOX AST test kit was used.

(E) Albumin [17]
RANDOX Albumin test kit was used.

(F) Bilirubin [18].
RANDOX Bilirubin kit was used.

(G) Alkaline Phosphatase (ALP) [19]
TECO Diagnostics, California, USA direct colorimetric ALP reagent kit was used.

(H) Gamma-glutamyl Transferase (γ-GT) [20]
RANDOX colorimetric (Kinetic Method) test kit was used.

(I) Urea [21]**.**
RANDOX Urease-Berthelot Colorimetric method kit was used

(J) Creatinine [22].
RANDOX Creatinine kit was used.

(K) Electrolytes (Na+, K+, Cl-, HCO3-) [23].
EA-1000B ISE electrolyte analyzer from Perlong Medical Equipment Company was used to measure these parameters.

(L) Platelets, WBC and Haemoglobin (Hb) [24].SYSMEX pocH-100i automated haematology analyzer was used for the measurement of platelets in the study population.

(M) Prothrombin Time (PT) [25]
AGAPE Diagnostics Switzerland Prothrombin Time kit was used.

Statistical Analysis

Data obtained was analyzed using Statistical Package for Social Sciences (SPSS) statistical software (Version 17 for windows) (SPSS Inc, Chicago, USA). Results were expressed as mean and standard deviation and were presented in tables.
Test of significance was done using Z- test, Pearson correlation coefficient statistics and Tukey HSD post HOC test. Values above 95% confidence limit were considered statistically significant.

III. RESULTS

Table 1 shows heavy metals and serum protein radicals concentrations measured in the two study groups. Highest levels of all the measured parameters were observed in residents in Imiringi community as compared to Odi Community. Z-test of the means of the two groups showed statistically significant differences ($p<0.05$).

Table 2 shows levels of the concentrations of the measured parameters in males and females in the two study areas. Highest levels of all the metals and protein radicals were observed in females in Imiringi. These observed differences in the two genders were only statistically significant in protein radicals. Females in Odi had the highest mean levels of cadmium and lead while males had the highest mean levels of mercury, selenium and protein radicals. These levels were only statistically significant in mercury and selenium.

Table 1: Heavy Metals and Protein Radicals Levels in the Study Populations.

Study Community	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radical
Imiringi (n = 200)	0.030 ± 0.006	0.026 ± 0.009	0.829 ± 0.206	10.794 ± 1.513	2.418 ± 0.893	54.55 ± 22.34
Odi (n = 200)	0.017± 0.01	0.004 ± 0.011	0.081 ± 0.098	0.929 ± 0.314	0.037 ± 0.064	20.44 ± 22.29
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S = Significant

Table 2: Heavy Metal and Protein Radical Levels in Males and Females.

	Imiringi				Odi			
	Males n = 84	Females n =116	F value	P value	Males n = 106	Females n = 94	F value	P value
Cd(ppm)	0.029 ±0.016	0.031±0.017	1.119	0.292	0.0176±0.014	0.018 ± 0.006	0.412	0.522
Cr(ppm)	0.025 ±0.01	0.026 ± 0.01	0.487	0.486	0.004 ± 0.003	0.004 ± 0.009	0.000	1.000
Hg(ppm)	0.817 ± 0.19	0.84 ± 0.225	0.579	0.448	0.109 ± 0.122	0.052 ± 0.06	16.89	<0.0001(S)
Pb(ppm)	10.652 ± 1.804	10.936±1.36	1.580	0.210	0.923 ± 0.212	0.935 ± 0.18	0.103	0.748
Se(ppm)	2.025 ± 1.09	2.811 ±1.32		<0.0001(S)	0.051 ± 0.07	0.022 ±0.05		0.02900(S)
Protein Radicals (ng/ml)	48.60 ± 20.40	40.55±21.68	7.056	0.007(S)	21.60 ± 19.20	18.40 ± 16.22	1.742	0.1884

S = Significant

The mean values of the measured heavy metals and protein radicals in the different age groups of Imiringi community are shown in table 3. Test of significance of these observed age induced variations using Tukey post HOC analysis are presented in table 4. The highest level of Cadmium was seen in residents 51- 60 years old (0.034 ± 0.005ppm) while the least mean concentration of Cadmium (0.020 ± 0.004ppm) was observed in residents 2 – 10 years old. These values were statistically significantly different at p<0.05 (Table 3). The highest mean value of chromium (0.032 ± 0.013ppm was recorded in the age group 31- 40 years while the least value (0.020 ± 0.003ppm) was recorded among residents 2 -10 years old. The difference in values was not statistically significant. Subjects 71- 80 years old, had the highest mean concentrations of mercury (0.971 ± 0.084ppm), Lead (11.85 ± 1.47ppm) and Selenium (3.14 ± 1.32ppm). The least mean values of Mercury (0.436 ± 0.142ppm), Lead (9.14 ± 0.81ppm) and Selenium (1.842 ± 0.31ppm) were measured among residents between the ages of 2 – 10 years old. These observed differences were statistically significant at p <0.05 (Table 4). It also shows that residents within the ages of 61 – 70 years had the highest mean concentration of protein radicals (71.95 ± 21.30) while the least level was observed in residents between the ages of 2 – 10 years. The observed difference was not statistically significant at p<0.05.

Table 3: Protein Radicals and Heavy Metals Levels in Subjects in Relation to Age in Imiringi Community..

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radicals (pg/ml)
2– 10(n=30)	0.020 ± 0.004	0.020 ± 0.003	0.436 ± 0.142	9.14 ± 0.81	1.842 ± 0.31	45.0 ± 14.24
11 –20(n=34)	0.025 ± 0.006	0.022 ± 0.08	0.835 ± 0.12	10.87 ± 1.82	2.043 ± 0.37	47.09 ± 17.24
21 – 30 (n=26)	0.028 ± 0.004	0.027 ± 0.006	0.803 ± 0.14	10.654 ± 1.31	2.054 ± 0.42	46.21 ± 17.10
31 – 40 (n=30)	0.029 ± 0.005	0.032 ± 0.013	0.820 ± 0.21	10.72 ± 0.85	2.098 ± 0.42	46.44 ± 19.44
41 – 50 (n=23)	0.030 ± 0.005	0.028 ± 0.009	0.685 ± 0.22	9.864 ± 0.77	2.20 ± 0.50	54.28 ± 20.39
51 – 60(n=25)	0.034 ± 0.005	0.026 ± 0.01	0.696 ± 0.23	10.24 ± 0.65	2.50 ± 0.87	63.41 ± 20.03
61 – 70(n=15)	0.032 ± 0.005	0.025 ± 0.008	0.74 ± 0.09	10.87 ± 0.55	2.94 ± 0.78	71.95 ± 21.30
71 – 80(n=17)	0.03 ± 0.01	0.024 ± 0.01	0.971 ± 0.08	11.85 ± 1.47	3.14 ± 1.32	62.01 ± 21.50

Table 4: Comparison of Heavy Metals and Protein Radicals Levels in Subjects in Relation to Age in Imiringi Community.

Age Range	Cd (P Value)	Cr (P Value)	Hg (PValue)	Pb(P Value)	Se (P Value)	Protein Radicals (P Value)
2-10 vs 11-20 2-10 vs 21-30	0.0092 <0.001(S)	1.0000 0.9946	<0.0001(S) <0.0001(S)	<0.001(S) <0.001(S)	0.9095 0.9155	0.1961 1.0000
2-10 vs 31-40	<0.001(S)	0.8737	<0.0001(S)	<0.001(S)	0.7695	0.9979
2-10 vs 41-50	<0.001(S)	0.9902	<0.0001(S)	0.3155	0.4571	0.3620
2-10 vs 51-60	<0.001(S)	0.9981	<0.0001(S)	0.0119(S)	0.0041(S)	<0.001(S)
2-10 vs 61-70	<0.001(S)	0.9998	<0.0001(S)	<0.001(S)	<0.001(S)	0.0003(S)
2-10 vs 71-80	<0.001(S)	0.9999	<0.0001(S)	<0.0001(S)	<0.0001(S)	0.0010(S)
11-20 vs 21-30	0.4398	0.9992	0.9959	0.9958	1.0000	0.2569
11-20 vs 31-40	0.0810	0.9397	1.0000	0.9995	1.0000	0.0345(S)
11-20 vs 41-50	0.0215(S)	0.9981	0.0237(S)	0.0301(S)	0.9839	1.0000
11-20 vs 51-60	<0.001(S)	0.9998	0.0391(S)	0.4328	0.1168	0.0235(S)
11-20 vs 61-70	0.0016(S)	1.0000	0.6007	1.0000	0.0002(S)	0.1839
11-20 vs 71-80	0.0532	1.0000	0.1190	0.0851	<0.001(S)	0.3633
21-30 vs 31-40	0.9976	0.9994	0.9999	1.0000	1.0000	0.9977
21-30 vs 41-50	0.9113	1.0000	0.2188	0.2540	0.9926	0.4262
21-30 vs 51-60	0.0036	1.0000	0.3099	0.9081	0.1950	<0.0001(S)
21-30 vs 61-70	0.3388	1.0000	0.9421	0.9990	0.0007(S)	0.0006(S)
21-30 vs 71-80	0.9423	1.0000	0.0322(S)	0.0218(S)	<0.001(S)	0.0017(S)
31-40 vs 41-50	0.9980	0.9999	0.0766	0.1337	0.9991	0.1000
31-40 vs 51-60	0.0222(S)	0.9981	0.1189	0.7845	0.2747	<0.0001(S)
31-40 vs 61-70	0.6777	0.9981	0.8012	0.9999	<0.0001(S)	<0.0001(S)
31-40 vs 71-80	0.9989	0.9943	0.0650	0.0304(S)	<0.0001(S)	<0.0001(S)
41-50 vs 51-60	0.2008	1.0000	1.0000	0.9492	0.7249	0.0447(S)
41-50 vs 61-70	0.9584	1.0000	0.9755	0.1501	0.0122(S)	0.2270
41-50 vs 71-80	1.0000	1.0000	<0.0001(S)	<0.0001(S)	0.0002(S)	0.4169
51-60 vs 61-70	0.9547	1.0000	0.9927	0.7024	0.4003	1.0000
51-60 vs 71-80	0.2991	1.0000	<0.0001(S)	0.0004(S)	0.0321(S)	0.9965
61-70 vs 71-80	0.9708	1.0000	0.0033(S)	0.2452	0.9865.	0.9999

The mean concentrations of protein radicals and heavy metals in the different age groups of Odi community are as shown in table 5. Test of significance (Tukey post HOC analysis) of these observed age induced variations are presented in table 6.The highest mean values of Cadmium (0.022 ± 0.01ppm), Mercury (0.097 ± 0.08) and Selenium (0.098 ± 0.08ppm) were recorded in residents between the ages of 71 – 80 years. Highest mean values of Chromium (0.038 ± 0.07ppm) and Lead (0.919 ± 0.25ppm) were recorded in residents 11 – 20 and 31 – 40 years respectively. Children 2 – 10 years old had the least mean values of Cadmium (0.012 ± 0.008ppm), Lead (0.502 ± 0.101ppm) and Selenium (0.045 ± 0.024). Least mean values of Chromium (0.001 ± 0.00) and Mercury (0.001 ± 0.001; Range: 0.00 – 0.004) were observed in residents 71 – 80, 21 – 30 and 11 – 20 years respectively. The observed differences in these

age groups were statistically different at P<0.05. The highest mean concentration of protein radicals (22.77 ± 19.20ng/ml) was recorded in residents 51 - 60 years old while residents that were between 2-10 years old recorded the least mean values (18.35 ± 22.29ng/ml). This variation was significantly different at p<0.05.

The correlation between measured metals and protein radicals in Imiringi community is presented in table 7.A weak and positive correlation was observed between Cadmium and protein radicals (r = 0.208). The observed correlation was statistically significant at p<0.005.

The mean values of liver function parameters that were measured are presented in Table 8. Residents in Imiringi community had the highest mean levels of all the parameters that were measured as compared to Odi community. Differences in the observed levels of these parameters were all statistically significant (p < 0.05).

Table 5: Protein Radicals and Heavy Metals Levels in Subjects in Relation to Age in Odi Community.

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radicals (ng/ml)
2-10(n=27)	0.012 ± 0.008	0.021 ± 0.004	0.016 ± 0.014	0.502 ± 0.101	0.045 ±0.024	18.35 ± 22.29
11- 20(n=36)	0.018 ± 0.004	0.038 ± 0.07	0.021 ± 0.02	0.612 ± 0.16	0.081 ± 0.011	20.37 ± 12.29
21-30(n=26)	0.016 ± 0.004	0.010 ± 0.002	0.001 ± 0.001	0.666 ± 0.17	0.056 ± 0.063	20.72 ± 18.20
31 - 40(n=32)	0.013 ± 0.003	0.012 ± 0.002	0.002 ± 0.002	0.919 ± 0.25	0.055 ± 0.05	20.59 ± 21.09
41- 50(n=20)	0.014 ± 0.002	0.009 ± 0.001	0.06 ± 0.04	0.900 ± 0.09	0.057 ± 0.004	19.31 ± 19.20
51- 60(n=21)	0.016 ± 0.02	0.006 ± 0.001	0.06 ± 0.04	0.90 ± 0.09	0.060 ± 0.04	22.77 ± 19.20
61- 70(n=20)	0.017 ± 0.01	0.004 ± 0.001	0.07 ± 0.08	0.864 ± 0.08	0.065 ± 0.07	21.13 ± 23.51
71- 80(n=18)	0.022 ± 0.01	0.001 ± 0.001	0.097 ± 0.08	0.818 ± 0.08	0.098 ± 0.08	20.27 ± 24.2

Table 6: Comparison of Heavy Metals and Protein Radicals Levels in Different Age Groups in Odi Community

Age Range	Cd (P Value)	Cr (P Value)	Hg (P Value)	Pb (P Value)	Se(P Value)	Protein Radicals (P Value)
2-10 vs 11-20	0.1275	1.0000	0.9997	0.0567	0.1371	0.9998
2-10 vs 21-30	0.7062	1.0000	0.8765	0.0011(S)	0.9950	1.0000
2-10 vs 31-40	0.9999	1.0000	0.8868	<0.0001(S)	0.9962	1.0000
2-10 vs 41-50	0.9940	0.9933	0.0067(S)	<0.0001(S)	0.9945	0.6262
2-10 vs 51-60	0.7631	0.9998	0.0056(S)	<0.0001(S)	0.9776	0.0081(S)
2-10 vs 61-70	0.5219	1.0000	0.0003(S)	<0.0001(S)	0.9052	0.0002(S)
2-10 vs 71-80	0.0052(S)	1.0000	<0.0001	<0.0001(S)	.0011(S)	0.0592
11-20 vs 21-30	0.9865	1.0000	<0.0001(S)	0.8256	0.5970	1.0000
11-20 vs 31-40	0.2664	1.0000	0.5338	<0.0001(S)	0.4698	1.0000
11-20 vs 41-50	0.7217	0.9964	0.5254	<0.0001(S)	0.7344	0.8424
11-20 vs 51-60	0.9908	1.0000	0.0146	<0.0001(S)	0.8353	0.0230(S)
11-20 vs 61-70	0.9999	1.0000	0.0122(S)	<0.0001(S)	0.9596	0.0007(S)
11-20 vs 71-80	0.7557	1.0000	0.0006(S)	<0.0001(S)	0.8294	0.1298
21-30 vs 31-40	0.8965	1.0000	0.0001	<0.0001(S)	1.0000	1.0000
21-30 vs 41-50	0.9943	0.9937	1.0000	<0.0001(S)	1.0000	0.7993
21-30 vs 51-60	1.0000	0.9998	0.0001(S)	<0.0001(S)	1.0000	0.0252(S)
21-30 vs 61-70	0.9999	1.0000	<0.0001(S)	0.0002(S)	0.9992	0.0008(S)
21-30 vs 71-80	0.3296	1.0000	<0.0001(S)	0.0005(S)	.0278(S)	0.1229
31-40 vs 41-50	0.9999	0.9820	<0.0001(S)	0.9998	1.0000	0.7965
31-40 vs 51-60	0.9233	0.9989	<0.0001(S)	0.9998	1.0000	0.0204(S)
31-40 vs 61-70	0.7436	1.0000	<0.0001(S)	0.8799	0.9978	0.0006(S)
31-40 vs 71-80	0.0129(S)	0.9998	<0.0001(S)	0.0461(S)	0.0105(S	0.1132
41-50 vs 51-60	0.9958	1.0000	1.0000	1.0000	1.0000	0.6894
41-50 vs 61-70	0.9585	0.9995	0.9937	0.9933	0.9997	0.0870
41-50 vs 71-80	0.0947	0.9999	0.0949	0.3865	0.0754	0.8989
51-60 vs 61-70	1.0000	1.0000	0.9933	0.9927	1.0000	0.8546

51-60 vs 71-80	0.3910	1.0000	0.0874	0.3637	0.1152	1.0000
61-70 vs 71-80	0.6437	1.0000	0.4438	0.9295	0.2757	0.8032

Table 7: Correlation between Heavy Metals and Protein Radicals in Imiringi Community

Protein Radicals	Cadmium		Chromium		Mercury		Pb(ppm)		Se(ppm)	
	R	P	R	P	R	P	R	P	R	P
	0.055	0.439	0238	0.001(S)	0.005	0.944	0.086	0.226	-0.044	0.536

Table 8: Mean and SD Values of Liver Function Parameters in the Study Populations

STUDY COMMUNITY n = 200	ALT (U/I)	AST (U/I)	G-GT (U/I)	ALP (U/I)	Albumin (g/l)	TP (g/l)	TB (µmol/l)	CB (µmol/l)
Imiringi	7.2 ± 1.6	10.5 ± 2	40.4± 3.3	24± 2	37.4 ± 3	63.3 ± 1	9.0 ± 0.08	2.8 ± 0.24
Odi	5.6 ± 1.3	9.5 ± 1.8	25.5 ± 4.2	22.3 ± 1	38.5 ± 2	63.8 ± 1.3	5.7 ± 0.1	2.0 ± 0.11
P Value	<0.0001(S)	<0.0001(S)	<0.001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

Table 9 shows the mean of concentration values of kidney function parameters that were measured in the residents of the two study groups. Inhabitants of Imiringi community had the highest levels of potassium (4.4 ± 0.4 mmol/l), creatinine (101± 4.0 µmol/l)) and urea 6.6 ± 0.9mmol/l). Residents of Odi had the highest mean levels of sodium (141 ± 2.2mmol/l), chloride (103 ± 3.6mmol/l) and bicarbonate (29.8 ± 4.4mmol/l). These differences were statistically significant in all the parameters. The mean values of the measured haematological parameters in the two study populations are presented in table 10. Imiringi residents had the highest mean prothrombin time (13.87 ± 1.14 sec). Odi residents had the highest mean levels of Hb (haemoglobin) (12.02 ± 1.5 g/dl), Total WBC (6.6 ± 0.63 $\times 10^9$/l) and platelet (208.5 ± 7.5 $\times 10^9$/l). All the measured differences in these parameters in the two communities were statistically significant ($p < 0.05$).

Table 9: Mean and SD Values of Kidney Function Parameters in the Study Populations.

Study Community	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mm0l/l)	Creatinine (µmol/l)	Urea (mmol/l)
Imiringi	139 ± 3.3	4.4 ± 0.4	100 ± 3.2	25..4 ± 3.0	101± 4.0	6.6 ± 0.9
Odi	141 ± 2.2	4.0 ± 0.5	103 ± 3.6	29.8 ± 4.4	97.7± 4.0	6.0 ± 0.7
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S = Significant

Table 910 Mean and SD Values of Haematological Parameters in the Study Populations.

STUDY COMMUNITY	Hb (g/dl)	WBC ($\times 10^9$/l)	Platelet ($\times 10^9$/l)	PT (Seconds)
Imiringi	11.3 ± 1.9	5.8 ± 0. 55	186.3 ± 6.3	13.87 ± 1.14
Odi	12.02 ± 1.5	6.6 ± 0.63	208.5 ± 7.5	12.74 ± 1.1
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

IV. DISCUSSION

The results of this study have shown increased and statistically significant levels of heavy metals in the blood samples of people living in the gas flaring community as compared to the control community. All the measured values of these metals in the two communities were above the recommended reference ranges except for selenium which was found to be within the reference range in the control population. This finding could be attributed to widespread pollution in the Niger Delta area which varies significantly depending on the predominant petroleum exploration activities in the particular location.

High levels of Cadmium, Chromium, Mercury, Lead and Selenium found in the blood samples of people living in the gas flaring community could be related to the fact that oxides of these metals are released in large amounts into the surrounding atmosphere continually during the process of gas flaring. Inhalation of oxides of these metals may result to increased metal uptake by the body and up to 10 - 40% retention of inhaled Cadmium in the human body had been reported [26][27][28]. These released oxides also mix with rain and are equally deposited on vegetation and water bodies in the community which are in turn consumed by the residents. This can account for another possible route of entry of these metals into human systems. Studies on surface and ground water samples obtained from some gas flared locations in Warri, Delta state, Nigeria were found to contain heavy metals at concentrations above the World Health Organization (WHO) maximum permissible limits [29]. This is due to the presence of metals and other substances in gas flare emissions. The results of physicochemical parameters obtained for soil, rain-water and air samples from gas flaring locations in Rivers state, Nigeria showed that gas flaring activities in the area have greatly impacted negatively on the inhabitants. The study showed that no meaningful human activity can take place in any of the gas flaring locations at radial distances less than 2 km away from flare points [30] This according to the researchers, is because of the high pollution loads imposed on these environments arising from increased pH of soil and acid rain concentrations (due to gas emissions), abnormal air temperature (due to flare radiation), heavy metal concentration and poor air quality due to flare emissions..

Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs. Long-term exposure can lead to gradually progressing physical, muscular, and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy. Repeated long-term exposure to some metals and their compounds may even cause cancer [26].

There was no clear cut pattern in the level of these measured metals in males and females. Though females in the gas flaring community (Imiringi) had the highest levels of all the metals, statistically significant difference was only seen in selenium. Some authors have reported significantly higher blood levels in males than in females for lead and cadmium [31], cadmium [32], lead [33], and nickel [34]. On the other hand, some other authors have reported no difference between heavy metals blood concentrations in men and women. [35] [31]

The effect of age differences on heavy metal bioaccumulation in the subjects shows different patterns of accumulation for different metals in different age groups. However, the results obtained show that bioaccumulation of the measured metals increased with age in the two study groups.

Statistically significantly increased levels of serum urea and creatinine observed in people living in the gas flaring area as compared to those in the control community suggests a higher risk of occurrence of renal impairment in them. This agrees with the findings by Egwurugwu *et al* [36] who also reported significantly increased serum levels of urea, creatinine, potassium, uric acid and inorganic phosphate in subjects exposed to pollutants of oil and gas. It has equally been demonstrated in

laboratory animals that crude oil causes destruction of the renal reserve capacity and also induced several pathological changes in the form of tubular necrosis [37]. The results also suggest a possible higher incidence of liver function impairment in people living in Imiringi community due to significantly higher levels of Gamma-GT, AST, ALT, ALP, total bilirubin and conjugated bilirubin measured in their blood samples. A similar research carried out on people living in areas prone to crude oil spillage showed similar results [38]. Several adverse alterations in haematological parameters affecting blood and the haemopoetic process negatively are known to be caused by crude oil exposure. [39][40].These negative changes could give rise to anaemia (aplastic), leukaemia and pancytopenia. The results of the measured haematological indices equally show that the residents in the areas where gas is flared may be prone to anaemia due to significantly lower levels of haemoglobin measured among them. They may also be prone to suppressed immunity as well as bleeding disorders as shown by the significantly lower levels of total white blood cells, platelets and longer prothrombin time measured in their blood samples as compared to the control community. A similar result was obtained in a research carried out on occupationally exposed oil workers in Barsa [39]. Experimental animals exposed to crude oil showed similar effects as well [40][41].

The observed high level of protein radicals and the positive correlation it had with cadmium is of significant importance because exposure to cadmium can cause a variety of pathological alterations in several organs and tissues as well as induce diabetic complications, hypertension and osteoporosis [42] Protein damage mediated by oxidation, protein adducts formation with advanced glycated end products and with products of lipid peroxidation, has been implicated during aging and age-related diseases, such as neurodegenerative diseases [43]. A hallmark of aging both at the cellular and organismal level is the accumulation of damaged macromolecules due to increased oxidative stress and failure of protein repair and maintenance systems[44][45]. Previous research has found that oxidative deterioration of biological macromolecules is primarily due to binding of heavy metals to the DNA and nuclear proteins [4]. Increased level of oxidatively modified proteins observed during aging and age related diseases could have deleterious effects on cellular and organ function. Increased levels of protein carbonyls have been observed in diseases, such as neurodegenerative diseases (amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases), cataractogenesis, systemic amyloidosis, muscular dystrophy, progeria, Werner's syndrome, rheumatoid arthritis, and respiratory distress syndrome [46]. Elevated levels of proteins modified by lipid oxidation products (4-hydroxy-2-nonenal: HNE, malondialdehyde) are associated with neurodegenerative diseases, iron-induced renal carcinogenesis, cardiovascular disease [47]. Elevated levels of protein glycation/glycoxidation end products (advanced glycation end products (AGEs) are associated with diabetes mellitus, neurodegenerative diseases, atherosclerosis, and Down's syndrome [47]. As seen in the study, exposure to gas flares which causes the accumulation of injurious substances could enhance these deleterious effects.

V. CONCLUSION

The findings of this study have shown that there is an increased level of heavy metals, protein radicals, liver enzymes such as AST, ALT, ALP, Gamma-GT, total bilirubin, conjugated bilirubin urea, creatinine and prothrombin time in the blood samples of people living in the gas flaring community. On the other hand, the levels of WBC and platelets were significantly lower in the test community than in the control community. These findings suggest that prolonged exposure to heavy metals could affect the health of inhabitants in the gas flaring area as elucidated above.

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